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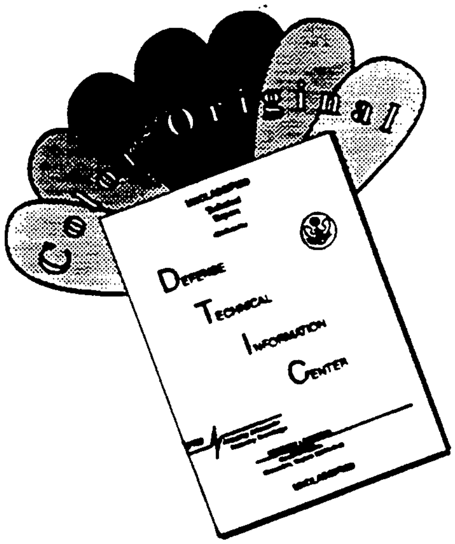
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THE USE OF HYDROXYAPATITE CEMENT IMPLANT IN HUMAN INTERPROXIMAL PERIODONTAL DEFECTS

A THESIS

Presented to the Faculty of

The University of Texas Graduate School of Biomedical Sciences

at San Antonio

in Partial Fulfillment of the Requirements for the Degree of

MASTER OF SCIENCE

By

Graig D. Brown, B.A., D.D.S

San Antonio, Texas

May 1996

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THE USE OF HYDROXYAPATITE CEMENT IMPLANT IN HUMAN INTERPROXIMAL PERIODONTAL DEFECTS

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DEDICATION

The years of my participation in the Residency in Periodontics have been unquestionably the most challenging and at the same time the most rewarding of my lifetime. Over the last three years, the most significant and unselfish sacrifices have been made by my best friend and loving wife Jean. Residency has a way of becoming an enormous distraction in your life and at times can become quite consuming. Jean's commitment to our marriage and friendship not to mention our family has often gone without any expression of appreciation or gratitude even though her efforts have contributed significantly to my success as well as the continued cohesiveness of our family. Jean has given and sacrificed from the heart without expecting anything in return. I dedicate this thesis to her ever mindful that this is little compensation for what I would consider the value of her contribution to my success in an extremely challenging residency. For the unconditional love of my sons Jonathan and Joshua, despite not always being there, I love you both with all my heart. I wish to thank both my parents, Rodney and Wanda Brown and Jean's Mother Joan Devitt for their love and support over the 14 fabulous years of our marriage. In memory of Jean's Father Edward ("Ned") Devitt, whose expressions of confidence in me and my potential for success have always and continue to be a driving force in my life, I am eternally grateful.

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I wish to thank my co-mentors Dr. Brian Mealey and Dr. Thomas Waldrop for trusting me with a project that, at it's onset, had the potential to impact on the clinical practice of periodontics and specifically periodontal regenerative therapy. Dr. Mealey is respectfully acknowledged for his insights in clinical research and for the impact his knowledge had on this project. For his surgical expertise and persistence in getting this project off the ground, I am grateful to Dr. Waldrop and feel very fortunate to have had the opportunity to work so closely with such a fine surgeon. The assistance and contributions of Dr. Pirka Nummikoski in "C.A.D.I.A. analysis" of clinical radiographs are respectfully acknowledged. The efforts of Dr. Sandra Bifano in statistical analysis of data were noteworthy and are greatly appreciated. Dr. David Cochran and Dr. Michael Brunsvold are acknowledged for the quality of their advice over the course of this project. I wish to thank Dr. Peter Costantino for his insights relative to the clinical behavior of Hydroxyapatite Cement and for sharing his knowledge following the completion of the clinical phase of this project. I am especially grateful to Dr. Brian Mealey for his critical review of this manuscript and for his reassurance regarding the value of the information attained through this endeavor.

THE USE OF HYDROXYAPATITE CEMENT IMPLANT IN HUMAN INTERPROXIMAL PERIODONTAL DEFECTS

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Thomas C. Waldrop

A new type of calcium phosphate cement (Hydroxyapatite Cement) has shown promise in the treatment of craniofacial defects. Sixteen patients with moderate to severe periodontal disease and 2 bilaterally similar vertical bony defects received initial therapy including scaling and root planing. Baseline clinical measurements recorded 4-weeks post-initial therapy included probing depth and clinical attachment levels utilizing a probing stent and conventional probe. In a split mouth design, all patients received the test material (Hydroxyapatite Cement) in one defect and either flap curettage only (negative control) or demineralized freeze-dried bone allograft (positive control) in the contralateral defect. The extent of the bony defect was determined during initial surgery and at 12 months during a reentry surgical procedure. Standardized radiographs taken at baseline and immediately prior to reentry at 12 months were evaluated by Computer-Assisted Densitometric Image Analysis (CADIA). All control sites healed without complications. Within six months of implant placement, 11 of 16 patients treated with Hydroxyapatite Cement had either exfoliated a large piece of the implant or the entire implant sequestered through the gingival sulcus. At all 16 test sites, a narrow radiolucent gap formed by one month post surgery at the initially tight visual interface between the radiopaque Hydroxyapatite Cement and walls of the bony defect. At 1 year, a mean probing depth reduction and clinical attachment gain were noted at sites treated with Hydroxyapatite Cement on the order of 1.6 ± 3.4 mm and 1.3 ± 2.4 mm, respectively. Minimal change at the apical base of defects (-0.1 ± 2.2 mm) and mean crestal resorption of 1.4 ± 1.7 were observed at test sites. Positive control sites (demineralized freeze-dried bone allograft) achieved a mean decrease in probing depth of 3.1 ± 1.4 mm along with a mean increase in clinical attachment level of 2.9 ± 1.6 mm. One year reentry measurements at sites grafted with demineralized freeze-dried bone suggested minimal change at the alveolar crest (0.2 ± 2.1 mm), apical defect fill (2.4 ± 3.1 mm), and greater than 70% defect resolution in 50% of patients. A probing depth reduction of 2.4 ± 2.0 mm and mean gain in clinical attachment of 1.4 ± 1.9 mm were observed for flap curettage controls. Mean crestal resorption (0.7 ± 1.5 mm), apical defect fill (1.1 ± 1.4 mm), and 50% defect resolution at 25% of sites were observed during reentry of flap curettage sites. CADIA revealed statistically significant differences between the three treatment modalities at various areas-of- interest. Mean density loss in the apical third of defects treated with Hydroxyapatite Cement was contrasted with mean density gains by both demineralized freeze-dried bone allograft and flap curettage. Significantly different mean density changes were also noted at the middle and crestal thirds of the defect when sites treated with Hydroxyapatite Cement were compared to flap curettage. No statistically significant differences were noted when mean density changes for sites treated with demineralized freeze-dried bone allograft were compared to sites treated with flap curettage, although the greater increase in density at flap curettage sites approached statistical significance at both the apical third of the defect and immediately below the bony cortex of the defect. Overall, there was a proportionally greater increase in density at sites treated with demineralized freeze-dried bone compared to flap curettage progressing from the base to the middle-third of the bony defect. Based on the findings of this study, there is no rationale available to support the use of Hydroxyapatite Cement Implant in its current formulation for the treatment of vertical intrabony periodontal defects.

TABLE OF CONTENTS

Title.......... i

Approval.......... ii

Dedication.......... iii

Acknowledgements.......... iv

Abstract.......... v

Table of Contents.......... viii

List of Tables.......... x

List of Figures.......... xi

List of Plates.......... xii

I. INTRODUCTION AND LITERATURE REVIEW.......... 1

- A. Autografts.......... 2
- B. Allografts.......... 3
  - 1. Freeze Dried Bone Allograft.......... 3
  - 2. Demineralized Freeze-Dried Bone Allograft.......... 4
- C. Alloplasts.......... 6
  - 1. Nonresorbable Ceramic Implant Materials.......... 7
  - 2. Resorbable Implant Materials (Ceramic and Non-ceramic).......... 9
  - 3. Hydroxyapatite Cement.......... 11

II. MATERIALS AND METHODS.......... 15

- A. Description of Human Subjects.......... 15

B. Data Collection.................... 18
C. Description of Surgical Procedures.................... 20
D. Radiographic Analysis.................... 22
E. Investigation Hypothesis.................... 26
F. Data Analysis.................... 26
G. Probing Reproducibility.................... 28
III. RESULTS.................... 30
A. Clinical Findings.................... 30
B. CADIA Results. 36
IV. DISCUSSION AND SUMMARY. 43
Literature Cited.................... 56
Vita.................... 74

LIST OF TABLES

Table 1 Randomization of treatment group combinations.. 16

Table 2 Baseline defect depth as measured in mm from the alveolar crest to the defect base.. 31

Table 3 Summary of statistical analysis of clinical parameters.. 34

Table 4 Comparison of clinical measurements (mean ± SD in mm) between experimental (HAC) and control treatments (F/C) & (DFDBA).. 35

Table 5 Gingival Index, Plaque Index, & Bleeding Index... 37

Table 6 Mean (± SD) change in defect density at various areas of interest for HAC, DFDBA and F/C sites as determined by CADIA... 39

Table 7 Summary of statistical analysis of CADIA... 41

LIST OF FIGURES

Figure 1 Change in defect density (CADIA value).. 40

LIST OF PLATES

Plate 1 Periodontal probing stent and set-up for mixing Hydroxyapatite Cement................. 64

Plate 2 Surgical treatment and reenty of a defect treated with DFDBA............................... 65

Plate 3 Steps in the surgical treatment of a bony defect with Hydroxyapatite Cement.......... 66

Plate 4 Reentry surgery at a site treated with Hydroxyapatite Cement................................. 67

Plate 5 Radiographic cone alignment device and customized film holder............................. 68

Plate 6 Radiograph of vertical bony periodontal defect with areas-of-interest....................... 69

Plate 7 Subtraction image of a defect utilized for CADIA... 70

Plate 8 Clinical exfoliation and sequestration of Hydroxyapatite Cement............................. 71

Plate 9 Radiographic observation of a defect treated with Hydroxyapatite Cement............. 72

Plate 10 Surgical treatment and reenty of a defect treated with flap curettage........................ 73

I. INTRODUCTION AND LITERATURE REVIEW

Periodontitis is characterized by inflammation of the supporting and surrounding tissues of the teeth leading specifically to loss of periodontal ligament and bone destruction.[1] Adult periodontitis is a chronic progressive disease. The loss of periodontal attachment (bone and periodontal ligament) around a tooth can progress to a severe stage resulting in tooth mobility, advanced bone loss, and eventual loss of teeth. Tooth loss results in need for costly prosthetic replacements. The ultimate goal of periodontal therapy is to provide a dentition that will function in health and comfort for the life of the patient.[2] Although prevention is the ideal defense against periodontitis, treatment and management of periodontitis requires continuous removal of the bacteria that inhabit the gingival crevice. The short term goal of periodontal treatment is to prevent further attachment loss. This goal has resulted in the evolution of a myriad of treatment modalities which aim to repair or regenerate lost periodontium that has been destroyed through the progression of periodontitis.

In the majority of periodontal diseases, control of supra- and subgingival plaque and their by-products is the most effective method for stabilization of the periodontium. A long term goal in periodontal therapy is to regenerate the periodontium to its prediseased state. To this end, a variety of treatment modalities have evolved in periodontics. To achieve this goal, periodontal surgical procedures are frequently required in order to repair or, ideally, regenerate lost periodontium. Successful surgical therapy should result in a periodontal crevice sufficiently shallow for patient maintenance. Currently, treatment modalities aimed at periodontal regeneration include bone graft or implant therapy, guided tissue regeneration with barrier membranes, or a combination of these materials. Current development of biologically inert, synthetic biomaterials (alloplasts) offers an additional array of materials for utilization in osseous periodontal defects with the intent to achieve regeneration.

In order to examine the efficacy and safety of alloplastic bone implant materials, the definition of some important terms needs to be established. **Repair** describes healing of a wound by tissue that does not fully restore the architecture or the function of the part.[1] **Regeneration** signifies reproduction or reconstitution of a lost or injured part.[1] **New attachment** defines the union of connective tissue or epithelium with a root surface that has been deprived of its original attachment apparatus. This new attachment may be epithelial adhesion and/or connective adaptation or attachment and may include new cementum.[1] A **graft** has been defined as: (1) any tissue or organ used for implantation or transplantation; (2) a piece of living tissue placed in contact with injured tissue to repair a defect or supply a deficiency; and finally (3) to induce union between normally separate tissue.[1] An **implant (oral)** specifically describes: (1) an alloplastic material or device that is surgically placed into the oral tissue beneath the mucosal or periosteal layer or within the bone for functional, therapeutic, or esthetic purposes; or indicates (2) the insertion of a graft or alloplastic device into the oral hard or soft tissues for replacement of missing or damaged anatomical parts, or for stabilization of a periodontally compromised tooth or group of teeth.[1] Tissue transferred from one position to another within the same individual is known as an **autograft**.[1] An **allograft**, on the other hand, is a graft between genetically dissimilar members of the same species.[1]

A. Autografts

Bone grafts, by definition, refer to tissues placed in osseous defects to stimulate bone formation or periodontal regeneration.[3] Autografts are grafts of tissue that are transferred from donor to recipient site in the same individual. Autografts that have been utilized in periodontal surgical procedures include osseous coagulum, cortical bone chips, and combinations of cortical and cancellous bone, cancellous-marrow from intraoral sites and cancellous-marrow from an extraoral donor site. The disadvantage of most of these materials is that the amount of donor material may not be sufficient for the size of the osseous defect. Other considerations with autografts include the quality and composition of the harvested material.

B. Allografts

Allografts refer to grafts of tissue that are transferred from a donor to a recipient site between genetically dissimilar individuals of the same species. These materials were developed because of the inherent problems of autogenous material, especially lack of an adequate amount of donor material. Bone allograft materials are a desirable alternative for the clinical periodontist since their availability is virtually unlimited. A major concern that has been expressed regarding allografts is the possibility of disease transmission. Protocols have been established for obtaining donor material at the level of tissue banks that are responsible for routine donor testing, tissue procurement and preparation.

1. Freeze Dried Bone Allograft

Freeze dried bone allografts (FDBA) were first used in periodontal therapy in the early 1970's.[4] The first clinical usage of FDBA was for orthopedic applications in 1950. Once the donor material has passed rigid testing that is required for accreditation by the American Association of Tissue Banks, the donor tissue is freeze dried. In a large clinical trial by Mellonig, *et al.* (1976), use of FDBA resulted in 64% of the defects showing greater than 50% bone fill.[5] In another study of 272 periodontal osseous defects treated with FDBA, 63% yielded 50% or better bone fill.[6] These results by study design were considered successful. The use of FDBA resulted in 64%, 60% and 63% of defects healing with greater than 50% bone fill in a three part field study.[5,6,7.] In part 4 of the latter study, bone fill greater than 50% was recorded on 67% of defects grafted with FDBA and 78% of defects grafted with FDBA plus autogenous bone.[7] Mellonig concluded that grafting with FDBA alone or FDBA combined with autogenous bone was effective for the treatment of periodontal osseous defects.[4] Several reports have considered the immunologic implications of allografts. Allografts have had the potential disadvantage of antigenicity of the donor material due to its origin from human donors. Current methods of processing the donor material, such as lyophilization, reduce the antigenicity in cortical bone. Both animal[8] and human studies[9] report markedly attenuated antigenicity associated with "cortical" FDBA. In a study by Quattlebaum, *et al.* (1988), FDBA failed to elicit a detectable first or second-set response of cytotoxic antibodies to FDBA.[9] The authors concluded that FDBA may be regarded as a graft material lacking clinically significant antigenicity when used to repair periodontal osseous defects in humans.[9]

2. Demineralized Freeze Dried Bone Allograft (DFDBA)

A study by Urist (1970) hypothesized that demineralization of freeze-dried bone allograft processed with hydrochloric acid exposes a bone inductive protein substrate known collectively as bone morphogenic protein (BMP).[10] Urist proposed that this protein would exert its inductive effect if the allogenic bone was demineralized effectively. This stimulation of new bone formation by the allograft BMP is called osteoinduction and was demonstrated by Urist and co-workers through numerous animal experiments.[10,11,12] The first use of DFDBA in human periodontal osseous defects was by Liben, *et al.*[13] Three sites responded with 4-10mm of new bone formation.[13] Mellonig studied 47 periodontal osseous defects in humans comparing open flap debridement with and without DFDBA to evaluate the efficacy of the graft material.[14] With surgical reentry, a mean bone fill of 2.6mm (65% defect fill) was obtained in DFDBA treated sites compared to 1.3mm (38% defect fill) with flap debridement only.[14] A histologic evaluation by Bowers, *et al.*(1989) compared the healing of intrabony defects associated with a diseased root surface with and without DFDBA.[15] In an analysis of 32 grafted and 25 nongrafted defects in 12 patients, the authors demonstrated histologically that DFDBA sites consistently formed new bone, new cementum, and new periodontal ligament.[15] Nongrafted defects in the same patients healed with a long junctional epithelium along the entire length of the exposed root surface.[15]

Although it has been shown that DFDBA appears to lack any significant antigenicity,[8,9] concern over the transmission of pathogens such as hepatitis B surface antigen and the human immunodeficiency virus (HIV) through allograft material still exists.[16,17] While the risk of transfer of such a disease is less than 1 in 8 million in DFDBA when proper tissue processing procedures are followed,[17] the use of an allograft requires a continued level of responsibility by procurement agencies and the dental profession in the wake of concerns over disease transmission. In spite of results such as those of Mellonig *et al.* (1992) which concluded that demineralization and treatment with a virucidal agent inactivated the HIV in spiked and infected bone, patient concerns may limit the appeal of allografts in the future.[18]

Questions regarding the osteoinductive capacity of allografts used in periodontal regeneration have recently been brought to light.[19] In a study evaluating the biological activity of protein extract prepared from commercially-obtained DFDBA and a fresh human specimen, it appeared that although BMPs retain protein with biological activity, the BMPs in DFDBA specimens are present at lower concentrations and have less ability to promote cell proliferation compared to extracts from fresh human bone.[20] Another study suggests that commercial DFDBA differs in size and ability to induce new bone formation between various bone banks and batches of bone allografts within banks.[21] DFDBA from 2 of 6 bone banks failed to induce new bone formation.[21]

C. Alloplasts

An alloplast is defined as an implant of inert material.[22] In view of the limitations of some of the other graft materials, alloplasts (synthetic foreign biocompatible materials) have been used in periodontal regenerative procedures in recent years. Despite both meticulous screening of donors and processing of human derived tissues, a fully synthetic graft material becomes attractive in this era of intense patient concern over disease transmission. The history of alloplastic materials in periodontal therapy includes many materials that offered no substantial benefit in periodontal repair including polymethylmethacrylate[23] and plaster of Paris.[24] On the other hand, hydroxyapatite (HA) and betatricalcium phosphate appear safe and may be clinically acceptable in obliterating various types of periodontal defects according to the Council on Dental Materials of the American Dental Association. Calcium phosphate based ceramic materials have a calcium to phosphate ratio similar to human bone in the form of hydroxyapatite (HA).[25,26] Definitive studies have shown that HA is not osteogenic[27] but rather osteoconductive in that it may serve as a scaffolding upon which host bone can grow. If macropores of 200-300 micrometers are fabricated into an HA implant, bone grows into the macropores, effectively bonding the implant material to bone (osseointegration).[27,28,29] The well documented biocompatibility as well as strong chemical and physical resemblance to bone mineral[30] are an enticement for the use of these materials as implants in the treatment of intrabony periodontal defects.

During the manufacturing of these calcium phosphate materials, ceramic powders are exposed to high heat under pressure (sintering). The manufacturer can control particle size, shape, density, pore size, and ultimately whether the material is porous or non-porous. Since the mid-1970's, preformed HA materials in various forms have been used in a variety of clinical applications in both medicine and dentistry.[28,31,32] These HA preparations have had limited applicability because they had to be preformed as hard materials.[28] Variation of the chemical and physical properties of calcium phosphate can influence the clinical behavior of these materials.[33] Ceramics under current use and review include tricalcium phosphate, non-porous hydroxyapatite (durapatite), and porous hydroxyapatite. Alloplasts are divided by composition into nonceramic and ceramic materials. Ceramic materials can be further divided into nonresorbable, partially resorbable, and resorbable materials. The following discussion of ceramic materials will include nonresorbable and resorbable ceramic implant materials.

1. Nonresorbable Ceramic Implant Materials

a. Non-Porous Hydroxyapatite (Durapatite). This material is a dense, pure, nonresorbable ceramic that has enjoyed widespread use as an implant material for the treatment of osseous periodontal defects. The most comprehensive research on non-porous hydroxyapatite has been reported by Yukna and coworkers. Comparative studies by this group provided significant information as they compared defects within patients treated with implants to debridement only controls at 6 month, 12 month, and 5 year reentry. Yukna, *et al.* (1985), reported that while available histologic data suggests non-porous HA ceramic does not induce new bone formation or new connective tissue attachment, it does appear to be a biocompatible filler that will support a dense connective tissue matrix over a long period of time.[34] Six of the original 13 patients in the Yukna study were followed under an active maintenance program for 5 years. Assessment of the clinical changes during the 5 year post-surgical period demonstrated that graft site attachment levels stayed the same 86% of the time compared to only 62% stability or improvement in debridement sites.[35] A consensus report of the World Workshop in Clinical Periodontics summarizes that non-porous HA does not resorb but remains as an inert encapsulated material within the connective tissue.[36] Non-porous HA may have some indications as a filler material but has no justification for use as a regenerative material in the treatment of periodontal osseous defects.[36]

b. Porous Hydroxyapatite. Extensive investigation of porous HA as an implant material in periodontal defects has shown that this material is capable of more favorable results than other alloplastic materials used to date.[37,38] Kenny, *et al.* (1985), reported that porous HA implanted in periodontal osseous defects produced statistically significant reduction in pocket depth, depth of osseous lesions, and gains in attachment levels compared to controls at a 6 month reentry.[37] Tissue samples from 3 patients taken at 3, 4, and 6 month intervals post-implantation with porous hydroxyapatite showed a narrow zone of bone formation along the walls of pores at 3 months, continued evidence of bone deposition with osteocytes, osteoblasts and collagen fibers apparent throughout the implant at 4 months, and pores with a primary composition of lamellar bone at 6 months.[38] Stahl and Froum found similar histologic results after treatment of osseous defects with porous hydroxyapatite.[39] Ossification of implant pores and the implant periphery were observed at 3 months and became pronounced at 12 months in the presence of a long junctional epithelial attachment (i.e. no new attachment was observed). In a multicenter study comparing porous HA to DFDBA, Oreamuno, *et al.* (1990) reported significantly greater clinical resolution of interproximal vertical periodontal defects treated with porous HA (Interpore 200) than those grafted with DFDBA.[40] A study by Bowen, *et al.* (1989) concluded that if regeneration of the periodontium is the desired goal, then DFDBA is still the material of choice.[41] If defect fill is the primary goal, then the result of implantation with porous HA (Interpore 200) equals that of DFDBA.[41] Since more favorable treatment outcomes supported by clinical and histologic findings have been reported with porous HA than with other alloplastic materials, the concensus report of the World Workshop in Clinical Periodontics recommended its continued use in defect repair.[36] Since the healing pattern associated with porous HA appears to be periodontal repair[39,42] and the long-term clinical implication of a graft material that does not resorb but fills a space is unknown, the benefit of porous HA may be limited.[4]

2. Resorbable Implant Materials (Ceramic and Non-ceramic)

a. Tricalcium Phosphate Implants (TCP). Tricalcium phosphate (TCP) is a porous form of calcium phosphate that is slowly resorbed. TCP is not a natural component of bone mineral.[30] In a study by Baldock *et al.* (1985), 13 osseous defects in 2 patients were filled with TCP.[43] Histologic findings included TCP particles which were apparently well tolerated and encapsulated by fibrous connective tissue, although the particles did not stimulate new bone growth and there was limited evidence of new attachment.[43] From the histologic observations of this study, the authors reported that the apparent radiographic fill observed was probably TCP material and not new bone growth.[43] In a histologic study of four TCP grafted sites in one patient, Bowers *et al.* (1986) reported that biopsy of sites reentered at one year revealed bone and osteoid formation around graft particles in all specimens.[44] There was also union of osteoid and ceramic particles in some regions.[44] Bowers concluded that: (1) TCP ceramic does appear to serve as a nidus for new bone formation in intrabony defects; (2) resorption and replacement of TCP may continue over several years and; (3) active bone formation was occuring supracrestally in 3 of 4 defects and in soft tissue coronal to the defect after one year.[44]

In a study by Stahl and Froum (1986), TCP implants did not appear to enhance osteogenesis or cementogenesis and there was limited evidence of new connective tissue root attachment.[45] A 1989 concensus report of the World Workshop in Clinical Periodontics concluded that TCP appeared to be resorbable; however, healing associated with TCP did not regenerate the periodontium.[36] The implant material did not have osteoinductive properties and served only as a filler material and, therefore, has no applicability for periodontal regenerative procedures.[36] A more recent study by Saffar *et al.* (1990) evaluated 5 biopsies obtained during reentry surgery of sites implanted with TCP at 16 to 40 months post implantation.[46] Saffar concluded that TCP: (1) promoted bone formation and; (2) actively resorbed before bone formation started and degraded at a later stage.[46] Saffar's study supported the findings of Bowers *et al.* (1986) that bone formation following TCP implantation occurred slowly over a period of years.[44] The slow nature of this reaction may explain the results of other studies which indicated no findings of bone formation or osteointegration.

3. Hydroxyapatite Cement (HAC)

A new type of calcium phosphate cement material which sets to HA *in vivo* when mixed with water has been developed at the Pafenbarger Research Center of the American Dental Association Health Foundation (ADAHF) by Brown and Chow.[29] HAC cement, a non-ceramic, is produced by direct crystallization of HA *in vivo* and does not require heating for the production of a structurally stable material.[29] The implant becomes a paste when mixed with water, blood, or saline which can be molded and contoured to fill osseous defects. Tests have shown that HAC is not as strong as ceramic HA but appears to have sufficient strength for reconstruction of non-stress bearing bone.[47]

a. Chemistry of HAC. The major components of HAC, tetracalcium phosphate and dicalcium phosphate dihydrate react in an aqueous environment to form HA.[29]

$$Ca_4\,(PO_4)_2 + CaHPO_4 \xrightarrow{H_2O} Ca_5\,(PO_4)_3OH$$

Pure HAC sets in vitro at 37°C (an isothermic reaction) within 15 minutes while maintaining a pH during setting of 6.5 to 8.0.[29] The chemical reaction is complete within 4 hours, producing HA as the only reaction product.[29] Under scanning electron microscopic examination, HAC is composed of small petal-like crystals which make up a microporous structure.[29] The bulk density of the set cement (HAC) is approximately 2.3 which corresponds to a pore content of 45% of total implant volume.[29] Dye penetration tests and scanning electron microscopic examination suggest an average effective pore diameter on the order of 2 to 5nm.[47,48,49] The size of these pores allows permeation of the set cement by ionic materials and dyes but prevents the passage of bacteria.[50]

b. HAC Implants in Craniomaxillofacial Defects. Recent investigations by Constantino and Friedman[50,51,52] have studied the use of HAC in the reconstruction and augmentation of various bone defects of the craniomaxillofacial skeleton in animal models. HAC disks were placed subperiosteally on the calvarium of cats.[50] The HAC disks demonstrated numerous foci of new bone formation within the spaces between disk and calvarium, bone ingrowth into the disks over surfaces in contact with the skull, and bone formation over the external disk surface not in skull contact but covered by periosteum.[50] Histologic response to HAC disks implanted in soft tissue was similar to the response seen with other commercially prefabricated forms of HA ceramic.[50] There was a lack of significant fibrous encapsulation, minimal inflammatory response, and an absence of foreign body giant cell formation to HAC.[50] Constantino and Friedman, *et al.* (1991) concluded that: (1) HAC appears to be more resorbable than ceramic HA and resorbability varies directly with implant surface area and; (2) HAC was found to be osteoconductive when implanted in subperiosteal pockets with bone growth onto the disk surface and into the implant material as it was slowly resorbed.[50]

c. Use of HAC in Dentistry. Since its development by Brown and Chow,[29] Hydroxyapatite Cement has been evaluated in several investigations. Functional evaluation of HAC in different *in vivo* applications such as a bone and periodontal cement and as a root canal filling material have been fostered by other early success.[47,48,49] One such study evaluated calcium phosphate cement (CPC) as a root canal sealer-filler in adult beagle dogs.[53] Histologic evaluation of the periradicular tissues of teeth sealed with the calcium phosphate cement showed tissue inflammatory responses similar to those of the well accepted control material gutta-percha.[53] Due to its previously proven biocompatibility,[48] the characteristic of tissue acceptance was considered beneficial in the event of overextension of the root canal sealer material.[53] A study of the clinical application of CPC for bone filling compared calcium phosphate cement to Apaceram (a commonly used ceramic hydroxyapatite material) in the lower jaws of dogs.[54] The investigators concluded from observed histo-pathological reactions that CPC had superior biocompatability and osteoconductivity than a commonly used material.[54] In a similar study, Fujikawa *et al.* (1993) compared CPC to Apaceram as an implant material in artificially formed periodontal bone defects in a dog model.[55] At 3 months, bone defects filled with CPC were covered with bone.[55] At 6 months, most of the implanted CPC had been converted to bone.[55] This was in contrast to bone defects filled with Apaceram which had interparticle spaces filled with fibrous connective tissue and bone and outward movement of some particles.[55] The authors concluded that since Apaceram is an adequate bone graft material clinically, present results suggest that CPC is an equally good or better material.[55]

The biocompatibility, osteoconductive, and resorptive properties of HAC suggest it has potential to function as a successful implant material in bone defects.[50] The unique property of HAC compared to ceramic HA is the relatively rapid replacement of the HAC by bone without significant loss of implant volume.[50] Based on efficacious and positive results of studies to date using HAC in skeletal cranial defects,[50,51,52] this study was proposed to examine efficacy of HAC in the regeneration of periodontal interproximal osseous defects in humans. The fact that this material is easily placed and contoured, biocompatible, resorbable, and osteoinductive make HAC an alloplastic implant material with the potential to overcome previous deficiencies of other alloplastic materials. The purpose of this investigation is to examine the efficacy of using hydroxyapatite cement (HAC) for the regeneration of human periodontal osseous defects of the alveolus.

II. MATERIALS AND METHODS

A. Description of Human Subjects

Sixteen systemically healthy patients (11 males and 5 females) with moderate to severe periodontal disease were recruited for this controlled clinical trial. The mean age of the study population was 40.4 years (female = 33.4; male = 43.6) and ranged from 23 to 60. The final study population of 16 included 8 patients in study group one (negative control group) and 8 patients in study group 2 (positive control group). Patients were selected who had at least two bilateral morphologically similar vertical intraosseous defects in the maxillary or mandibular molar or pre-molar region. Molar and premolar teeth with class 2 or 3 furcation involvement or mobility class III were not included in the study. The probing depths of these defects after the initial or hygienic phase of periodontal therapy were greater than or equal to 6 mm. Periodontal charting, including pocket depths and clinical attachment levels, and recent periapical radiographs were used to identify suitable subjects for the study. Each patient received initial therapy which included oral hygiene instructions, scaling and root planing, and occlusal adjustment when necessary. All patients entered into the study had achieved at least an 80% level of plaque control (Modified O'Leary plaque index or % of plaque free surfaces) effectiveness prior to beginning the surgical phase of therapy.[56,57] In addition to a determination of the severity of the periodontal defect prior to surgery through a variety of periodontal probing attachment measurements, the extent of the defect was determined during initial and reentry surgery.

Patients with bilaterally similar defects were randomly assigned to one of two treatment groups utilizing a split mouth design (Table 1). Group 1 patients (negative control group)

Table 1. RANDOMIZATION OF TREATMENT GROUP COMBINATIONS

	combination 1 (group 2)	combination 2 (group 1)	combination 3 (group 2)	combination 4 (group 1)
right side	HAC	HAC	DFDBA	F / C
left side	DFDBA	F / C	HAC	HAC

HAC = Hydroxyapatite Cement; **DFDBA** = Demineralized freeze-dried bone allograft; **F/C** = Flap curettage.

received the Hydroxyapatite Cement (HAC) test material (Hydroxyapatite Cement, Osteogenics, Inc., Richardson, TX.) in one defect and flap curettage at the contralateral defect. Group 2 patients (positive controls) received the test material (HAC) in one defect and demineralized freeze-dried bone allograft (DFDBA) in the contralateral defect (American Red Cross Tissue Services, Washington, DC). The DFDBA particle size was 200-300µ. HAC was terminally sterilized by the manufacturer.

1. **Recruitment of Human Subjects.** Twenty-five patients were recruited for the study as they presented for treatment at Wilford Hall Medical Center. All subjects were recruited by qualified investigators and understood and signed a detailed Informed Consent form approved by the FDA and Medical Law (Wilford Hall Medical Center). The Informed Consent form explained in detail the procedure to be performed, the expected benefits, the risks involved, and alternative procedures. Each signed consent form was validated by Medical Law, Wilford Hall Medical Center. Consent was obtained from all participants by the investigator who performed the surgical procedure. The patient pool from which patients were recruited consisted of local active duty military members and their adult dependents, retired military members, and dependents of retired military members.

2. **Patient Exclusion Criteria.** Patients were excluded from participation in the study if they presented with a history of need of critical restorative dentistry procedures, documented chronic steroid use, previous periodontal surgery in the past 24 months, abnormal calcium metabolism, significant systemic disease including metabolic bone disease or uncontrolled endocrinopathy, history of a recent (within 3 months) infection regardless of site, history of immunologic abnormalities, documented renal disease, current pregnancy, radiation treatment

involving the jaws, cardiovascular disease which would preclude elective periodontal surgery, children 18 years of age and under, and patients who had taken antibiotics within the last 3 months.

3. Specimens. Various soft tissue specimens curetted from chronic defects during the defect debridement component of surgical procedures were submitted for biospy on an as needed basis for informational purposes only. No hard tissue biopsy specimens or sections were obtained.

B. Data Collection

All data collected during the study were recorded on a series of Investigation Data Forms. The principal examiner in the study (GDB) was responsible for all data collection. The principal examiner was blinded to previous data acquisitions at successive data collection follow-up appointments. Although a second examiner for the collection of data would have been optimal, personnel limitations did not allow for an additional examiner during the investigation. Clinical indices were utilized for the evaluation of gingival soft tissue health. The Gingival Index (GI) of Loe and Silness[58] and plaque index (PI) of Loe[59] were used to evaluate signs, symptoms and etiologic factors associated with periodontal disease. The presence of bleeding on probing was also noted as a bleeding index (BI).[60] Clinical parameters were evaluated approximately four weeks after initial therapy, at the baseline examination.[61] Indices including GI, PI, and BI were determined weekly for the first month following the initial surgery, every 2 weeks post-op in the 2nd and 3rd months, and monthly during post-op visits from months 4 to 12. Periodontal probing depths and probing attachment levels were recorded at baseline, 6, and 12 months. Records were maintained by the principal investigator on each subject with data sheets maintained by number to protect patient confidentiality.

1. **Clinical and Surgical Probing Measurements.** All probing measurements were accomplished using acrylic stents and a conventional hand held periodontal probe (PCPUNC15 - Hu-Friedy®, Chicago, IL) marked at 1 mm increments. Customized acrylic occlusal stents were fabricated and stored on stone casts coded in sealed plastic containers. Each patient had their own individual PCPUNC15 probe which was used for all measurments throughout the study. A lateral groove cut into the acrylic stent controlled probe placement into the deepest portion of the periodontal pocket and intrabony defect (Plate 1A). The groove served as a fixed reference point for both mesio-distal placement and apico-coronal angulation of the probe. Probing depths and osseous measurements were made from both the facial and lingual aspects of the interproximal site. Following surgical determination of the deepest portion of the defect (facial versus lingual), all follow-up measurments were made at that position. If the facial and lingual measurements were equal, all follow-up measurements were recorded from the facial. All probing measurements in the study were made by the principal investigator with the benefit of 2.5 power magnification loops (Designs For Vision, Inc. Ronkonkoma, NY). A manual probing force of approximately 25 grams was used for clinical probing depth measurements which were recorded to the nearest 0.5 mm. Clinical measurements examined included both probing depth and clinical attachment levels. Osseous measurements recorded at initial and reentry surgery included: (1) CEJ to BC (the distance from the cementoenamel junction to the bony alveolar crest); (2) CEJ to BD (the distance from the CEJ to the base of the bony defect; and (3) BC to BD (the distance from the bony alveolar crest to the base of the bony defect at its deepest point). The described technique

and measurements are considered the standard for evaluating regenerative procedures.[62] In addition to measurements recorded during initial and reentry surgeries, clinical measurements were recorded approximately 4-weeks post initial preparation before surgery (presurgical clinical measurements) and postoperatively at 6 and 12 months. The clinical measurements included: (1) probing depth; (2) amount of gingival recession; and (3) clinical attachment level. The data obtained from these measurements were utilized to evaluate the clinical healing response at test and control sites.

C. Description of Surgical Procedures

Patients were sedated utilizing a continuous infusion intravenous conscious sedation technique incorporating diazepam (Valium®, Roche Products Inc., Manati, Puerto Rico) and nalbuphine hydrochloride (Nubain®, Dupont Pharmaceuticals, Manati, Puerto Rico). Local anesthesia was established with Xylocaine 2% with 1:100,000 epinephrine (Astra, Astra Pharmaceutical Products, Inc., Westborough, MA.). On the day of the initial surgery, both defects were treated in all 16 patients. Defect exposure was accomplished by reflecting full thickness mucoperiosteal flaps. Following debridement of granulation tissue and root surfaces at the surgical site, osseous defects were characterized by a series of measurements previously described. Intramarrow penetration to allow egress of pluripotential marrow reticular cells was performed.[63] Bony defects were treated according to previous treatment group assignment (Table 1) with 1 of 3 possible treatment modalities: (1) flap curettage only (F/C); (2) defect debridement plus HAC implant (HAC); or (3) defect debridement plus osseous grafting with Demineralized Freeze-Dried Bone Allograft (F/C plus DFDBA). DFDBA reconstituted with sterile water was placed to the height of the remaining osseous walls of grafted bony defects. Plate 2 (A-F) demonstrates the treatment of a positive control site with DFDBA.

Preparation of HAC for implantation included the incorporation of HAC powder with sterile water which was mixed on a glass mixing slab until a malleable "putty-like" consistency was achieved (Plate 1B). In bony defects treated with HAC, the material was carried to the defect with a double ended amalgam carrier (Arnel Inc., Hempstead, NY.), condensed into the defect with an amalgam condenser (Ladmore #3, Hu-Friedy®, Chicago, IL), and contoured until the implant reached the height of the remaining osseous walls of the defect. Plates 3 and 4 illustrate the treatment sequence at a test site implanted with HAC. Flaps were approximated in a conventional manner, with every effort made to achieve primary soft tissue closure of all surgical sites. External mattress sutures were used over study defects in order to avoid disturbing graft or implant materials. No surgical dressings were used. Patients and their escorts received postoperative instructions. Ibuprofen 800 mg was prescribed as an analgesic (Motrin®800mg, The Upjohn Company, Kalamazoo, MI.). All patients received doxycycline hyclate 100 mg daily (Vibramycin® Hyclate, Pfizer Inc., New York, NY) for a period of 14 days beginning on the day of surgery. In addition, chlorhexidine gluconate 0.12% (Peridex®, Proctor and Gamble, Cincinnati, OH.) was prescribed for twice daily rinsing for one month post surgery. Patients were instructed to rinse with 1/2oz. for 30 seconds twice daily. Patients were seen for follow-up visits weekly for the first month post-operatively including suture removal at 1 week. Postoperative follow-up continued every 2 weeks during postoperative months 2 and 3 and monthly thereafter until reentry surgery at 12 months. Oral hygiene measures including twice daily sulcular brushing and flossing were reinstated at 1 month. Oral hygiene instruction, plaque control, and evaluation of tissue response was accomplished at all postoperative follow-up visits.

As surgical reentry procedures are considered to provide the most reliable data for evaluation of regenerative materials and procedures, reentry of all treated defects was accomplished approximately 12 months following the initial surgical procedure. The evaluation of osseous implant procedures via osseous measurements is a compromise between highly invasive histological measurements of soft and hard tissue biopsies, and use of soft tissue measurements only.[64] The reentry surgical procedure involved less time and trauma in comparison to the primary surgical procedure. At the time of the 12 month reenty surgery, residual osseous defects were debrided and retreated with either no further treatment, an osseous resective approach, osseous grafting, or a combination technique utilizing guided tissue regeneration with a barrier membrane and osseous grafting. Treatment depended upon the morphology of the residual osseous defect. The protocol used for the reentry was identical to the approach described for the initial surgical procedure providing exposure, visualization, evaluation and the opportunity to retreat any residual osseous defect. All bony measurements previously described were repeated at reentry.

D. Radiographic Analysis

Standardized vertical bitewing radiographs were obtained utilizing vertical bitewing film holders (Rinn Corporation, Elgin, IL) customized for each patient with polyvinylsiloxane impression putty (Regisil, Caulk® Dentsply®, Milford, DE.) The bite registration material was added to the film holder in order to capture an occlusal registration (Plate 5). A cone alignment device (Rinn Corporation, Elgin, IL) customized with three parallel guide pins (Plate 5) allowed consistent and reproducible alignment of the x-ray cone perpendicular to the radiographic film. The customized film holders were maintained in sealed and labeled plastic containers. All radiographs were exposed on Kodak Ultraspeed Standardized periapical radiographic film (Eastman Kodak Company, Rochester, NY., USA). Standardized radiographs of each treatment site were exposed immediately prior to initial surgery, immediately post-op, 6 months post-op, and 12 months post-op, immediately prior to surgical reentry. All radiographs were exposed by a single dental x-ray unit (Gendex GX 1000, Gendex Corporation, Milwaukee, WI) at 65KVP, 15mA, and 30 impulses in order to produce optimum film density for computer image analysis. Exposed films were processed in an automatic processing system (System 30 DX Allied Photo Products, Fischer Industries, Inc., Geneva, IL) closely monitored daily for temperature and status of developer chemicals.

Standardized radiographs were analyzed by a single investigator (GDB). The image analysis system was composed of an Intel 80486 AT/bus personal computer (Lane Systems; San Antonio, TX) and a non-interlaced high resolution monitor (Mitsubishi Diamond Scan, HC 3925 ATK; Mitsubishi Electronic Corp, Nagasagi, Japan) with Microsoft Windows™ operating system. The analysis was performed with an image analysis software program known as Computer Aided Radiographic Evaluation (CARE).[65] The radiographs acquired during the study were positioned on a light stage where a calibrated CCD video camera (Dage-MTI CCD-72; Dage-MTI Inc., Michigan City, IN) captured a video image of each radiograph. Video images of each radiograph were then converted to a 640 x 480 pixel digital image with a framegrabber (VFG 100; Imaging Technology Inc., Woburn, MA). For this study, a 12 month follow-up radiograph of each study osseous defect was aligned with a stored baseline image using a real-time subtraction method for evaluating sequential pairs of standardized radiographs, and all images were saved on optical disk (Panasonic LF-7010; Matsushita Electric Industrial Co., Ltd. Osaka, Japan) utilizing an optical disk cartridge (Panasonic LM-D501W; Matsushita Electric Industrial Co., Ltd. Osaka, Japan). The CARE program[65] incorporates realtime subtraction capabilities and is based on a modification of the RADWORKS Program For Direct Digital Radiography.[66] The two x-rays required for the analysis were a baseline/pre-treatment film and a 12 month pre-reentry exposure of each treated osseous defect. The CADIA system can measure changes in bone density as small as 5%.[67] This compares to conventional radiography which requires a 30% change in bone mineral content to be detectable by the human eye.[68] Clinical trials of the CADIA system have been used to assess density changes at bone sites exposed to periodontal surgery.[69] The CADIA system has been shown to be capable of identification of surgically induced bone loss with a sensitivity of 82%, a specificity of 88% and a diagnostic accuracy of 87%.[69] Inherent to the subtraction process is a certain amount of inaccuracy associated with the set of sequential radiographs being analyzed. Although a highly accurate method for capturing standarized sequential radiographs has been reported,[70] the equipment required includes a cephalostat which limits the use of these methods to facilities which possess such technology. If radiographic images cannot be completely aligned, areas of differing gray levels (structured noise) may appear on the subtracted image, making it difficult to distinguish from gray level variations due to actual bone changes.[71] Background noise level in subtraction images was established by calculating the standard deviation of pixel values observed in a non-treatment area-of-interest (AOI) in the body of the mandible or maxilla as far apical as possible from the osseous defect. This value was determined for each set of radiographs. "Significant density change" (± 2 standard deviations = 95% confidence interval) was chosen by doubling the standard deviation.[72] This method was employed in order to exclude 95% of the difference that could be accounted for by normal image variation (noise) in the system. Prior to conducting the CADIA analysis, a non-parametric histogram matching program was used to adjust for differences in overall gray tone levels between images.[73] Five AOIs were selected for each intrabony defect using a preformed 16 x 16 pixel box, each representing 0.4 mm^2 of area (Plate 6). AOI number one (AOI-1) was positioned at the base of the radiographic bony defect against the visible cortex without contacting tooth root structure. AOI number two (AOI-2) was positioned in the middle third of the defect. The location of AOI number 3 (AOI-3) was in the coronal third of the defect approximately 1 mm apical to the adjacent alveolar crest. AOI number four (AOI-4) was positioned immediately apical to AOI-1 below the bony cortex of the defect's base or lateral wall but not in contact with tooth root structure. The final AOI (AOI-5) was positioned an arbitrary distance from the base of the defect in an area where, theoretically, there should have been no surgically associated change in bone density. Since AOI-5 was a nontreatment area of interest, CADIA values were used for informational purposes only. Every attempt was made to consistently position AOIs, although some variation occurred between pairs of radiographs due to variation in the morphology of the defects such as decreased bony width approaching the defect base. Radiodense residual HAC prohibited the preferential positioning of AOIs in the middle and coronal thirds of some defects, resulting in a more apical orientation for AOI-2 and AOI-3 in some cases. Changes in osseous density between the baseline radiographs and the pre-reentry radiographs were calculated. These differences were quantitatively expressed as the CADIA values representing average density increases and decreases within specific AOIs.

The images stored digitally for CADIA could also be retrieved for subtraction radiography as a subjective visual aid (Plate 7).

E. Investigation Hypothesis

The clinical responses for evaluation of efficacy of techniques in the present study included: (1) actual bony defect fill; (2) crestal alveolar resorption; (3) defect resolution (overall treatment effect); (3) change in probing pocket depth; (4) change in clinical attachment level and; (5) marginal soft tissue recession. The hypothesis tested by this study included: (1) the Null Hypothesis (H_0): There are no significant differences in the treatment outcomes of clinical new attachment gain, probing depth reduction, marginal soft tissue recession, alveolar crest resorption, apical defect fill, and percent defect resolution when defects are treated with HAC or demineralized freeze-dried bone allograft (DFDBA); and (2) an Alternate Hypothesis (H_1): There is significantly greater bone defect fill, gain in clinical new attachment, and probing depth reduction when osseous defects are implanted with HAC compared to flap curettage only. Likewise, use of HAC will result in less alveolar crest resorption and less marginal soft tissue recession compared to flap curettage.

F. Data Analysis

Data were expressed as means ± standard deviation of 32 defects in 16 patients. No data points were missing. Study outcome variables included:

Marginal Soft Tissue Recession (mm) = (final pre-reentry recession) - (Baseline recession);

Clinical Attachment Gain = (Baseline CAL) - (Pre-reentry CAL);

Probing Depth Reduction = (Baseline PD) - (Pre-reentry PD);

Amount of Apical Defect Fill (mm) = (Initial CEJ to BD) - (Reentry CEJ to BD);

Crestal Resorption = (Reentry CEJ to BC) - (Initial CEJ to BC);

% Defect Resolution = [(BC to BD initial - BC to BD final) ÷ BC to BD initial] x 100.

Both the clinical soft and hard tissue data of this study violate the normality assumption. Failure to achieve a normal distribution was the result of an unforeseen small sample size which occurred following termination of the study. A power analysis completed prior to the initiation of this study indicated that a final n of 20 was required to provide power of .80 to statistically detect a difference of .8 standard deviations or more (ie. 30% or more in the amount of the osseous defect filled). Previous studies of various treatment modalities used for repair or regeneration of periodontal osseous defects show differing defect fill percentages of 30%,[74] differing probing attachment level measurements on the order of 1-2 standard deviations,[40] and differing alveolar crest measurements of approximately .75 standard deviation.[40] Reduction of statistical power in this study was a function of both a smaller than originally planned study population and Bonferroni adjustments to the p-value (level of significance) due to multiple paired comparisons of data. All statistical calculations were performed using SPSS for Microsoft® Windows$_{TH}$ Release 6.1 statistical analysis software.[75]

To test the hypotheses in this study, statistical analysis of data from clinical soft and hard tissue measurements and CADIA included comparisons between groups using the Mann Whitney U- Wilcoxon Rank Sum Test. The Wilcoxon Matched-Pairs Signed-Ranks Test was used for paired comparisons of within group data. Alpha level was set at .05 and power at .80. Bonferroni correction was applied as appropriate for multiple comparisons. The alpha levels were selected to minimize the chance occurrence of significance resulting from the large number of comparisons among the treatment parameters evaluated. Corrected alpha levels were determined with a Bonferroni adjustment which divides the initial alpha by the number of comparisons made. Four comparisons of various clinical measurements were made within groups 1 and 2 resulting in a corrected significance level of $p<0.0125$. Within group comparisons of 4 specific intraosseous measurements resulted in a corrected level of significance ($p<0.0125$). Four comparisons for both intraosseous and clinical measurements were made between group 1 controls (flap curettage sites) and group 2 controls (DFDBA sites) resulting in a corrected significance level of $p<0.0125$ for intraosseous and for soft tissue parameters. In statistical analysis of CADIA data, 4 comparisons between and within treatment groups were made resulting in corrected alpha levels of $p<0.0125$.

G. Probing Reproducibility

Intraexaminer reproducibility with a conventional manual probe probe (PCPUNC15 - Hu-Friedy®, Chicago, IL) was evaluated prior to initiation of this study. The primary examiner (GDB) recorded probing depths and clinical attachment levels on three patients with moderate adult periodontal disease two times at an interval between 7 and 10 days. The patients, already entered into the primary study, had a minimum of 25 teeth including at least one first or second molar in each quadrant and no third molars. Measurements were estimated to the nearest 1 mm. At the mid-facial and mid-lingual points of each tooth, measurements were made with the probe parallel to the long axis of the tooth. For interproximal measurements, the probe was positioned inside the line angle of the tooth as close as possible to the interproximal contact point while attempting to keep the probe as close as possible to the long axis of the tooth. Probing depth was recorded first followed by attachment level determination. Both probings were recorded without the benefit of local anesthesia and the examiner was blinded to prior probing records.

In total, 12 quadrants containing 77 teeth were included. Teeth were probed at 6 sites per tooth for a total of 462 sites evaluated. Approximately 12% of measured sites had probing depths deeper than 3 mm and 27% of sites had attachment levels deeper than 3 mm. The percent agreement within ± 0.0 and ± 1.0 mm variation were determined. Agreement between first and second probing series resulted in a 60.4% exact match (± 0.0 mm variability) for probing depth and 57.6% exact match for clinical attachment level measurements. If an allowance for variability is made (± 1.0 mm variation between the first and second probings), reproducibility increased to 97.4% for probing depth and 93.7% for clinical attachment levels. By defining sites as deeper sites (greater than 3 mm), reproducibility for probing depth and attachment level within ± 1.0 mm variation was 90.7% and 92.0%, respectively.

III. RESULTS

A. Clinical Findings

Baseline defect characteristics for experimental, positive (DFDBA) and negative (flap curettage) treatments are illustrated in Table 2. Although the means and standard deviations of baseline defect depths described in Table 2 are descriptive and not statistically meaningful, mean defect depths were similar prior to treatment. Although most of the osseous defects were composed of 2-, and 3-walls, some defects had a 1-walled component. Defect morphology overall included 3-, 2-, 1- and combination-walled interproximal intrabony defects. All 32 surgical sites healed initially without any signs of clinical infection or adverse tissue reactions. Immediately prior to the one year surgical reentry, soft tissue measurements were repeated. Thirty-two defects (16 pairs in 16 patients) were reentered at approximately one year post-initial surgery. The original 32 defects included 5 maxillary and 27 mandibular defects. Two surgical test sites experienced postoperative sequestration of the entire HAC implant by three weeks. Both of these defects were located at the distal surface of terminal mandibular second molars. As the postsurgical period following the initial treatment progressed, sequestration of the HAC implant was common. By 6 months post-op, 11 of 16 patients had either exfoliated a large piece of the HAC implant or the entire implant was sequestering through the gingival sulcus associated with the treated defect (Plate 8). In all 16 sites treated with HAC, a narrow radiolucent gap formed within one month of surgical implantation at the initially tight visual interface between HAC and the bony cortex along the radiographic wall of the bony defect (Plate 9). All control sites healed uneventfully and no patients experienced postoperative complications in either defects

Table 2. BASELINE DEFECT DEPTH AS MEASURED IN MM FROM THE ALVEOLAR CREST TO THE DEFECT BASE.

Patient number	BC-BD (HAC)	BC-BD (F/C)	BC-BD (DFDBA)
1	5	8	
2	5	5	
3	5	5	
4	4	4	
5	4.5	4	
6	4	3	
7	4.5	3	
8	3	7	
9	3		3.5
10	3		2
11	5		3.5
12	5.5		9
13	1.5		4.5
14	4		8.5
15	7		4
16	4		4
Mean ± SD	**4.5 ± 1.3**	**4.9 ± 1.8**	**4.9 ± 2.5**

treated by flap curettage alone or with flap curettage plus DFDBA. Further inclusion of patients initially recruited into this study and awaiting surgical treatment was discontinued at 16 when it became apparent that the clinical outcome of HAC implantation into periodontal vertical bony defects was sequestration/exfoliation of the implant. Due to post-surgical clinical findings related to HAC which the investigators judged to be detrimental, the study was discontinued with a total of 16 patients having completed the first phase of surgical therapy. As patients entered into the study, they were surgically treated over a condensed period of time. By the time that unfavorable clinical findings such as implant sequestration appeared to be the norm, 16 patients had completed the initial surgical phase of the investigation.

Non-parametric analysis of both soft and hard tissue measurements and treatment outcomes revealed no statistically significant differences within or between treatment groups 1 and 2. Baseline probing and intraosseous defect depth measurements were not significantly different between or within groups. No statistically significant differences were detected for any clinical measurements including change in probing depth, change in clinical attachment level, or amount of marginal soft tissue recession either between groups (flap curettage vs. DFDBA) or within groups (HAC vs. flap curettage or HAC vs. DFDBA). Comparison of marginal gingival recession between control groups (flap curettage vs. DFDBA) approached statistical significance ($p = 0.0235$) with greater recession noted at sites treated with flap curettage. No statistically significant differences were detected for any intraosseous parameter including amount of actual defect fill, total crestal alveolar resorption, or percent defect resolution either between treatment groups or within treatment groups. Although comparisons failed to reach statistical significance for any of the parameters analyzed, a type II error was working due to the small sample size.

Table 3 summarizes the results of statistical analysis of within group soft and hard tissue comparisons and between group soft and hard tissue comparisons, respectively. Means and standard deviations of soft and hard tissue parameters were computed and are shown in Table 4 for their descriptive value, as the data were not statistically meaningful. Clinically significant trends were evident and although not statistically significant, comparison of various treatment endpoints provided information clinically relevant to the utilization of these treatment modalities in periodontal regenerative procedures. In sites treated with HAC, probing depth reduction and clinical attachment gain at 1 year were 1.6 ± 3.4 mm and 1.3 ± 2.4 mm, respectively. Gingival recession was minimal at sites treated with HAC (-0.8 ± 1.1 mm). Reentry osseous measurements at HAC sites revealed 1.4 ± 1.7 mm of crestal resorption and minimal change at the base of bony defects (-0.1 ± 2.2 mm) when compared to baseline measurements (Table 4). Resolution of defects treated with HAC (mean 29.2% ± 32.7) was primarily a result of resorption which occurred at the alveolar crest.

Results expressed as means and standard deviations for both control groups in the study were favorable (Table 4). For the eight defects treated with DFDBA, a mean decrease in probing depth of 3.1 ± 1.4 mm was noted along with a mean clinical attachment gain of 2.9 ± 1.6 mm and minimal recession (-0.1 ± 0.6 mm). One year reentry measurements at DFDBA sites appeared to indicate minimal crestal resorption (-0.2 ± 2.1 mm), favorable apical defect fill (2.4 ± 3.1 mm), and a mean defect resolution of 41.9% ± 42.9%, with greater than 70% defect resolution in 50% of the patients (defects) grafted. Gingival recession of 1.0 ± 1.1 mm was observed at negative control (flap curettage) sites. Probing depth reduction of 2.4 ± 2.0 mm and a mean gain in clinical attachment level of 1.4 ± 1.9 mm were recorded at the eight sites treated with flap curettage.

Table 3. SUMMARY OF STATISTICAL ANALYSIS OF CLINICAL PARAMETERS

Within Group Comparisons

Clinical Parameter	Groups Compared	Calculated P value
baseline PD	HAC vs. F/C	$p = 1.000$ NS
gingival recession	HAC vs. F/C	$p = .5625$ NS
Δ PD	HAC vs. F/C	$p = .2969$ NS
Δ CAL	HAC vs. F/C	$p = .8438$ NS
baseline defect depth	HAC vs. F/C	$p = .8125$ NS
crestal resorption	HAC vs. F/C	$p = .3954$ NS
apical defect fill	HAC vs. F/C	$p = .3047$ NS
total defect resolution	HAC vs. F/C	$p = .4990$ NS
baseline PD	HAC vs. DFDBA	$p = .4375$ NS
gingival recession	HAC vs. DFDBA	$p = .1250$ NS
Δ PD	HAC vs. DFDBA	$p = .4063$ NS
Δ CAL	HAC vs. DFDBA	$p = .3047$ NS
baseline defect depth	HAC vs. DFDBA	$p = .5156$ NS
crestal resorption	HAC vs. DFDBA	$p = .0469$ NS
apical defect fill	HAC vs. DFDBA	$p = .1172$ NS
total defect resolution	HAC vs. DFDBA	$p = .3281$ NS

Statistical test: Wilcoxon Matched-Pairs Signed Ranks Test ($n = 8$ for group 1 & group 2) (NS = not statistically significantly different)

Level of Significance for multiple paired comparisons: Alpha was set at .05 and power at .80. Bonferroni correction applied as appropriate for multiple comparisons within groups. Corrected alpha for multiple paired comparisons $p< 0.0125$.

Between Group Comparisons

Clinical Parameter	Groups Compared	Calculated P value
baseline PD	F/C vs. DFDBA	$p = .5215$ NS
gingival recession	F/C vs. DFDBA	$p = .0235$ NS
Δ PD	F/C vs. DFDBA	$p = .8729$ NS
Δ CAL	F/C vs. DFDBA	$p = .4579$ NS
baseline defect depth	F/C vs. DFDBA	$p = 1.000$ NS
crestal resorption	F/C vs. DFDBA	$p = .3973$ NS
apical defect fill	F/C vs. DFDBA	$p = .3973$ NS
total defect resolution	F/C vs. DFDBA	$p = .5249$ NS

Statistical Test: Mann-Whitney U - Wilcoxon Rank Sum W Test (NS = not statistically significantly different)

Level of Significance for multiple paired comparisons: Alpha was set at .05 and power at .80. Bonferroni correction applied as appropriate for multiple comparisons between groups. Corrected alpha for multiple paired comparisons: $p< 0.0125$.

Table 4. COMPARISON OF CLINICAL MEASUREMENTS (MEAN ± SD IN MM) BETWEEN THE EXPERIMENTAL (HAC) AND CONTROL TREATMENTS (F/C) & (DFDBA)

Soft tissue measurements

Treat-ment	PD(i)	PD(f)	CAL(i)	CAL(f)	REC (t)	PD (Δ)	CAL (Δ)
HAC	6.9 ± 1.8	5.3 ± 2.3	7.1 ± 1.8	5.8 ± 2.0	-0.8 ± 1.1	1.6 ± 3.4	1.3 ± 2.4
DFDBA	8.4 ± 2.0	5.0 ± 1.5	8.1 ± 1.5	4.9 ± 1.9	-0.1 ± 0.6	3.1 ± 1.4	2.9 ± 1.6
F/C	7.1 ± 2.1	4.3 ± 1.5	7.0 ± 1.8	5.3 ± 1.9	1.0 ± 1.1	2.4 ± 2.0	1.4 ± 1.9

HAC (n = 16) DFDBA (n = 8) F/C (n = 8) (i = initial; f = final; t = total)
PD = probing depth; CAL = clinical attachment level; REC = gingival recession.
Marginal soft tissue recession: (+) = actual marginal soft tissue recession.
ΔPD = probing depth reduction
ΔCAL = clinical attachment gain

Hard tissue measurements

Treat-ment	BC-BD(i)	BC-BD(f)	CEJ-BD(i)	CEJ-BD(f)	crestal resorption	apical defect fill	% defect resolution
HAC	4.3 ± 1.3	2.9 ± 1.6	7.8 ± 2.2	7.8 ± 2.6	1.4 ± 1.7	-0.1 ± 2.2	29.2 ± 32.7
DFDBA	4.9 ± 2.5	2.7 ± 2.1	8.1 ± 2.6	5.7 ± 2.2	-0.2 ± 2.1	2.4 ± 3.1	41.9 ± 42.9
F/C	4.9 ± 1.8	3.1 ± 1.1	8.4 ± 2.0	7.4 ± 1.8	0.7 ± 1.5	1.1 ± 1.4	32.3 ± 23.8

HAC (n = 16) DFDBA (n = 8) F/C (n = 8) (i= initial; f = final)

Note: crestal resorption (+) = crestal resorption; (-) = crestal apposition.

Mean crestal resorption of 0.7 ± 1.5 mm, apical defect fill of 1.1 ± 1.4 mm, and defect resolution of 32.2 ± 23.8% were observed during reentry of flap curettage sites. Two of 8 flap curettage sites had greater than 50% defect resolution.

Soft tissue status at the sites being evaluated was monitored from baseline to various post-treatment intervals. Gingival index (GI),[58] plaque index (PlI),[59] and bleeding index (BI)[60] were compared in order to evaluate general trends in the status of the gingival soft tissues for various treatments. The data for GI, PlI, and BI were inadequate to conduct a meaningful statistical analysis so this information is presented for descriptive purposes only. Gingival inflammation at the surgical site as measured by the gingival index (GI) decreased from the pre-treatment baseline to the 12 month follow-up for all three treatments (Table 5). Decrease in the GI was most pronounced for positive control sites (DFDBA) with a mean decrease from a GI of 2.4 ± 0.5 at baseline to 0.7 ± 0.5 at the 12-month reentry. The plaque index showed proportionately similar decreases for all three treatments over the 12 months of the study (Table 5). The percentage of treatment sites that converted from bleeding to non-bleeding was most pronounced at positive control (DFDBA) sites; however, a decrease in the overall percentage of sites exhibiting bleeding upon probing was noted for both test (HAC) and negative control (flap curettage) sites (Table 5).

B. CADIA

CADIA was performed for 16 defect pairs (32 total defects) using 4 treatment areas-of-interest. Choice of statistical analysis was limited by the small sample size of the study. The D'Agostino Omnibus was used to test the normality of data. In order to justify the normality assumption of the D'Agostino Omnibus, graphics were utilized to determine if the data of this small sample were normally distributed. Although the D'Agostino Omnibus suggested acceptance

TABLE 5. GINGIVAL INDEX,[60] PLAQUE INDEX,[61] & BLEEDING INDEX.[62]

Treatment Group	Gingival Index	Plaque Index	Bleeding Index (% of sites BOP)
HAC (bl)	2.2 ± 0.4	1.3 ± 0.8	100%
HAC (f)	1.7 ± 0.5	0.9 ± 0.6	69%
DFDBA (bl)	2.4 ± 0.5	1.0 ± 0.5	100%
DFDBA (f)	0.7 ± 0.5	0.5 ± 0.5	0%
Flap Curettage (bl)	2.3 ± 0.5	1.5 ± 0.5	100%
Flap Curettage (f)	1.5 ± 0.5	1.0 ± 0.9	50%

bl = baseline index   f = final/post-treatment (12 month pre-reentry) index
BOP = bleeding on probing

of the normality assumption, data in general were skewed for all three treatments over the four radiographic areas-of-interest. The authors elected to reject the normality assumption and proceed to non-parametric analysis of data.

Mean density changes resulting from treatment with HAC, flap curettage, and DFDBA are noted in Table 6 and are graphically depicted in Figure 1 for AOIs-1, 2, 3 and 4. Although the means are not statistically meaningful, clinically significant trends are apparent with test sites (Hydroxyapatite Cement) registering a mean density loss while the means for both control treatments reflected density increases. Standard deviations for mean CADIA values were large for all treatment modalities at all areas-of-interest. The Wilcoxon Matched-Pairs Signed-Ranks Test was utilized for analysis within treatment groups 1 and 2. Statistically significant differences within group 1 (HAC compared with flap curettage) were noted at AOI-1 (p=0.0117), AOI-2 (p=0.0117) and AOI-3 (p=0.0117). Within group 2 (HAC compared to DFDBA), a statistically significant difference was noted at AOI-1 (p=0.0117). Between group comparisons were analyzed with the Mann-Whitney U - Wilcoxon Rank Sum Test. No statistically significant differences were noted between groups (flap curettage compared to DFDBA) at any AOI although differences approached significance at AOI-1. Table 7 provides a summary of the results of statistical analysis for comparisons made both within and between treatment groups for radiographic density change as measured by CADIA at various areas-of-interest.

Correlation between the clinical parameter apical defect fill and radiographic density change as measured by CADIA was determined with a Pearson Correlation test. Thirty-two data points for the parameter apical defect fill measured during reentry surgery were compared with 32 data points each for radiographic density change as measured by CADIA at AOI-1 and AOI-2 to

TABLE 6. MEAN (± SD) CHANGE IN DEFECT DENSITY AT VARIOUS AREAS OF INTEREST FOR HAC, DFDBA AND F/C SITES AS DETERMINED BY CADIA.

Treatment	n	AOI-1 (apical)	AOI-2 (mid-defect)	AOI-3 (crestal)	AOI-4 (defect cortex)
HAC	16	-14.3 ± 12.6	-15.8 ± 18.5	-17.5 ± 19.6	-15.1 ± 16.5
DFDBA	8	2.3 ± 4.0	4.9 ± 7.2	1.6 ± 6.6	-2.6 ± 9.3
F/C	8	18.9 ± 15.4	15.5 ± 16.1	13.0 ± 19.9	6.0 ± 11.6

Figure 1. Radiographic change in defect density (mean change ± standard error).

Change in defect density measured in CADIA units at 4 areas-of-interest were compared for Hydroxyapatite Cement implant, DFDBA and flap curettage. Positive values indicate an increase in density and negative values represent density loss or density decrease.

CHANGE IN DEFECT DENSITY
(mean change ± standard error)

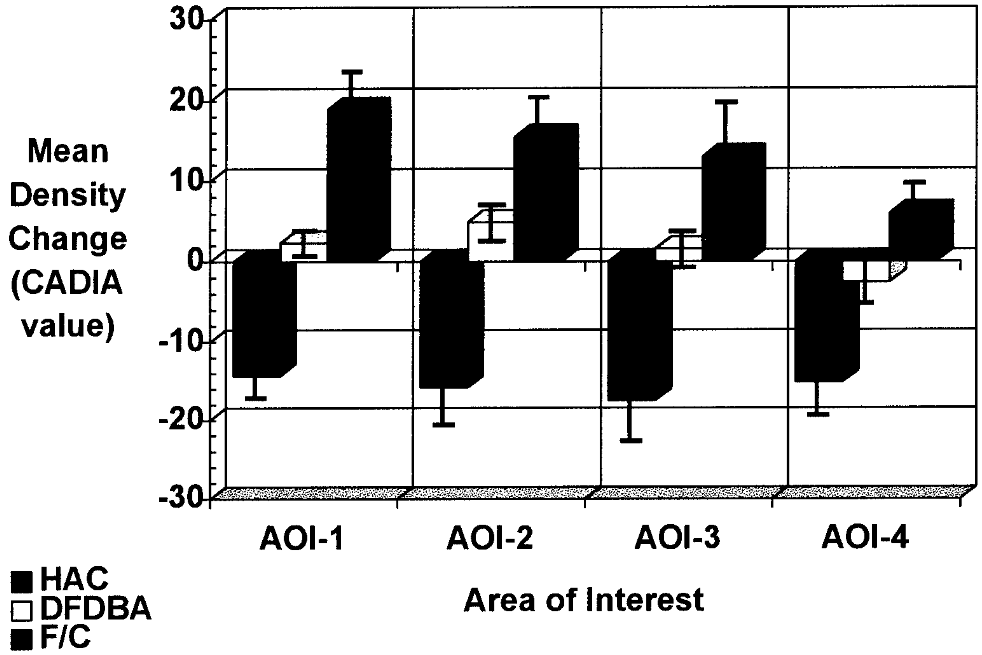

TABLE 7. SUMMARY OF C.A.D.I.A. STATISTICAL ANALYSIS

Within Group Comparisons

Radiographic Parameter	Groups Compared	Calculated P value
AOI-1	HAC vs. F/C	$p = 0.0117^{\dagger}$
AOI-2	HAC vs. F/C	$p = 0.0117^{\dagger}$
AOI-3	HAC vs. F/C	$p = 0.0117^{\dagger}$
AOI-4	HAC vs. F/C	$p = 0.0173$ NS
AOI-1	HAC vs. DFDBA	$p = 0.0117^{\dagger}$
AOI-2	HAC vs. DFDBA	$p = 0.0251$ NS
AOI-3	HAC vs. DFDBA	$p = 0.0630$ NS
AOI-4	HAC vs. DFDBA	$p = 0.0630$ NS

Statistical test: Wilcoxon Matched-Pairs Signed Ranks Test ($n = 8$ for HAC vs. F/C and HAC vs. DFDBA) († = statistically significant; NS = not statistically significantly)

Level of Significance for multiple paired comparisons: Alpha was set at .05 and power at .80. Bonferroni correction applied as appropriate for multiple comparisons. Corrected alpha for multiple paired comparisons $p < 0.0125$.

Between Group Comparisons

Radiographic Parameter	Groups Compared	Calculated P value
AOI-1	F/C vs. DFDBA	$p = 0.0209$ NS
AOI-2	F/C vs. DFDBA	$p = 0.1152$ NS
AOI-3	F/C vs. DFDBA	$p = 0.0927$ NS
AOI-4	F/C vs. DFDBA	$p = 0.0820$ NS

Statistical Test: Mann-Whitney U - Wilcoxon Rank Sum W Test († = statistically significant; NS = not statistically significantly)

Level of Significance for multiple paired comparisons: Alpha was set at .05 and power at .80. Bonferroni correction applied as appropriate for multiple comparisons. Corrected alpha for multiple paired comparisons $p < 0.0125$.

determine if a significant correlation between these outcome variables exists. When radiographic density change at AOI-1 was compared to apical defect fill, the Pearson Correlation of $r^2 = 0.05$ ($r = 0.22$) was not significant ($p = 0.36$). When apical defect fill was compared to radiographic density change at AOI-2, the correlation $r^2 = 0.05$ ($r = 0.23$) failed to reach statistical significance ($p = 0.38$).

IV. DISCUSSION AND SUMMARY

HAC appears to be sufficiently structurally stable for reconstruction and augmentation of non-stress-bearing portions of the craniofacial skeleton.[50] When in contact with viable bone, the cement is replaced, at least in part, with osseous tissue, without a significant loss of implant volume.[50] This property is unique when compared with commercial ceramic HA preparations. Bone formation observed over the external surface of HAC implanted subperiosteally is generated by the periosteum itself,[50] which has significant endogenous osteogenic potential.[76] Vascularized bone ingrowth enhances the long-term stability of the reconstructed area and offers increased resistance to infection.[51] Despite a lack of osteogenesis, a direct chemical bond forms between the HAC implant and bone without intervening fibrous tissue when HAC is placed in non-stress bearing areas of the craniofacial skeleton.[50] HAC has been implanted by ENT-Craniofacial Plastic Surgeons as part of a craniofacial reconstruction protocol testing HAC in defects of the human craniofacial complex.[77] Recurrence of a frontal sinus mucocele in one case necessitated block section of HAC implant and adjacent bone.[77] The human block section of HAC and adjacent bone clearly demonstrated the capacity of HAC to osseointegrate with bone adjacent to the frontal sinus.[77] When HAC is placed into contact with viable bone or periosteum, it is replaced by bone as the implant is resorbed.[50] This bonding of implant to bone is referred to as osseointegration.[27,28] The first clinical trial applying HAC to cranial defect repair involved 100 patients with a total of 182 defects reconstructed with pure HAC.[77] At 42 months, 75% of the HAC implants were in contact with the paranasal sinuses or mastoid.[77] HAC appeared to be functionally nonresorbable on the basis of serial axial, coronal, and three-dimensional CT scans and on direct intraoperative inspection of those cases that were re-operated for secondary reconstructive procedures.[77] While bone appeared to osseointegrate with cement at its periphery, larger implants such as those used in frontal bone repair appeared to be both volume and contour stable at more than 3 years.[77]

Success of HAC implant in craniofacial reconstruction has prompted interest in other areas of medicine and dentistry. The concept of an implant material with chemical characteristics similar to natural bone and physical properties which allow the cement to be molded, shaped and sculpted warranted clinical investigation to determine its utility in the regeneration of periodontal osseous defects. A structurally stable implant that is incorporated into surrounding bone is certainly a goal of both craniofacial and periodontal reconstructive surgery. In an electron microscopic evaluation of the osteoclastic features of multinucleated giant cells responding to synthetic hydroxyapatite jaw implants, the development of the ruffled border-clear zone system, indicative of new bone deposition, was dependent upon the physiochemical properties of the hydroxyapatite.[78] The failure of HAC implant to adhere and ultimately integrate to the walls of the periodontal osseous defect in the present study was likely due to biophysical characteristics of the implant cement which resulted in biomaterial failure. Why HAC failed to osseointegrate in these defects is unclear but three possible mechanisms should be considered.

The first is the lack of sufficient flexural stress resistance of set HAC. Unfortunately, the biophysical demands on the material are vastly different in the craniofacial complex in comparison to those of the periodontal supporting apparatus and specifically the alveolar bone. Reconstruction of a craniofacial defect involves an area of non-stress bearing bone. Although the alveolar bone may in general be non-stress bearing, the periodontal vertical bony defect is always associated with the root surface of a natural tooth. With the presence of even normal occlusal forces, the periodontal defect can be described as anything but non-stress bearing. Although porous-surfaced orthopedic implants have been designed for fixation by bone ingrowth, there is clinical evidence that this does not always occur.[79] Experimentally, bone ingrowth in orthopedic and endodontic implants in beagle dogs has been described in the presence of some movement, although very small (up to 28 μ), while excess movement (up to 150 μ) can result in attachment by mature connective tissue ingrowth.[79] Although a 10 N axial load in humans caused a 28 μ tooth displacement,[80] the difference in periodontal ligament compliance between dog and man is unknown. Another study reports that loads of a few Newtons caused apical displacement of teeth relative to their alveoli of about 10 to 100μ.[81] Based on the results of these studies, it would be reasonable to speculate that it may require only 50 to 100 μ of micromovement to result in failure of the HAC implant to osseointegrate. Stability at a site during healing is essential if early angiogenesis is to progress to bone formation, remodeling of damaged bony cortex, and ultimately intimate contact of bone to the surface of an implant material. Regardless of the type of implant system being considered, early tissue infiltrate within the pores of an implant with sufficient initial stability will differentiate to bone by either direct bone formation within the pores or appositional bone growth from the adjacent bone into the porous region.[82] Flexure of a tooth upon its periodontal ligament under normal occlusal loads results in flexure of the tooth root against the set HAC implant. If osseointegration of the HAC implant to the bony walls of the periodontal defect is dependent on immobility of the implant-bone-tooth interface, failure to achieve ingrowth or even bone-to-implant contact is almost certain.

It is noteworthy that sequestration of HAC implants in two cases involving defects at the distal of mandibular second molars occurred within three weeks of surgical placement. HAC implanted into interproximal defects at locations other than distal to mandibular second molars tended to exfoliate at time points considerably later postoperatively. Second molars are the most common teeth demonstrating nonfunctional contacts, with a 49% prevalence of such contacts.[83] Although some studies report that teeth with contact in nonfunction have significantly greater mobility,[84] other studies have reported that mobility was not significantly influenced by nonfunctional contacts.[83] In the present study, teeth with nonfunctional contacts were adjusted when appropriate. Material failure of HAC within periodontal intraosseous defects may be related to the mechanical characteristics of the cement. The set cement may lack sufficient flexural resistance to accommodate normal tooth movement associated with occlusal function. As the tooth is flexed under normal occlusal load, a wedge effect may lead to ejection of the set HAC implant from the vertical defect. Since HAC does not have adhesive properties conducive to adherence to the walls of the bony periodontal defect and lacks flexural resistance, the failure of the material to integrate to bone in this periodontal application is likely associated with tooth micromovement.

The second factor that must be considered is the lack of sufficient porosity in the set HAC to allow bone in-growth to occur. SEM analysis of set HAC implant revealed a pore size on the order of 5 to 8 μ (Plate 8). Although the spaces between an implant or graft particles may be critical, overall pore size of a set implant or an interparticular space (pore size) large enough to allow for migration and ingrowth of cells, blood vessels and bone is essential.[85] It has been demonstrated that pores larger than 100 μ are necessary for rapid ingrowth of vascularized fibrous tissue.[86] Although SEM analysis of HAC cement revealed the consistent presence of a microporous surface, the pore size range of 5 to 8 μ is probably inadequate to support angiogenesis and vascular ingrowth of tissues required to support osseointegration. Retention of Hydroxyapatite Cement Implant is dependent on some degree of osseointegration. Of the 16 sites in the present study treated with HAC, a radiographic gap indicating failure of the implant material to osseointegrate was apparent within one month of implantation at all 16 sites (Plate 9). The majority of HAC implanted into vertical periodontal osseous defects in this study sequestered before any of the remodeling observed in craniofacial defects had likely occurred.[51,52] One interesting finding worth noting was the mean apical defect fill (1.9 ± 1.9 mm) at sites where HAC sequestered within 4 weeks of the initial placement. This is in contrast to an actual mean increase in the depth of the defect measured at the defect base (-0.95 ± 1.7 mm) at sites where the HAC implant did not sequester within the first month postoperatively but rather sometime between 1 and 6 months postoperatively. Although these means are not statistically meaningful, retaining the nonintegrated HAC implant seemed to be increasingly detrimental to the long term outcome of the initial surgical procedure. From a descriptive standpoint, apical defect fill at test sites that sequestered the implant in the early stages of postoperative healing (1.9 ± 1.9 mm) was very similar to the results achieved by flap curettage controls (1.1 ± 1.4 mm).

A third possible mechanism contributing to the clinical failure of HAC in intrabony periodontal defects is bacterial contamination and colonization of the implant surface and micropores. Based on previous studies, [50,51,52,77,87] HAC appears to be capable of undergoing bony substitution. The microporous structure of HAC is large enough to allow bacterial contamination prior to tissue ingrowth which could effectively prevent bony substitution. HAC placed to augment edentulous mandibular ridges from a sterile extraoral approach in a nonhuman study resulted in both osteoconductive bone formation at the surface of the HAC implant and osteoinductive bone formation more than a 1 cm away from the cement/bone interface under the periosteum.[87] This was in contrast to findings of only osteoconductive activity and no new bone formation at control sites which were treated with a 75/25 ratio of porous hydroxyapatite (Interpore 200) and demineralized freeze-dried bone.[87] Although the dogs in this study were subject to a soft diet, the HAC implants were subject to some degree of movement as a result of normal occlusal load.[87] Implant integration, bony replacement and new bone formation under adjacent periosteum in this study[87] as well as implant integration adjacent to an implant placed in the frontal sinus[77] may have been dependent upon a closed wound environment. The sterility of the closed wound conceivably provided the implant surface and microporous structure the appropriate environment for bony substitution/replacement or osseointegration similar to what occurs as normal lamellar bone undergoes remodeling. Conversely, in the current study HAC implants were not in such a closed environment, and bacterial contamination via the healing sulcus may have resulted in bacterial colonization of the implant surface. Bacterial plaque contamination of the HAC implant would have certainly compromised any chance for bony integration between implant and host bone.

The results observed at DFDBA and flap curettage control sites are consistent with results reported in the periodontal literature. Reports on repair of adjacent medium and wide 3-wall intrabony defects following open debridement procedures in humans have described bone fill amounts on the order of 2.55 mm.[88] This compares to mean apical defect fill of 1.1 ± 1.4 mm at flap curettage sites in the present study. Plate 10 illustrates the initial appearance of an osseous defect in the present study treated with flap curettage and the appearance of the residual defect at reentry. Several studies have evaluated the treatment potential of DFDBA for the treatment of intrabony defects.[14,89] Quintero *et al.* (1982) utilized a reentry procedure at 4-6 months to determine the amount of bone fill in 1-, 2-, and 3-walled defects.[89] They reported an average bone fill of 65% (2.4 mm) along with a mean increase in probing attachment level of 1.9 mm.[89] A direct comparison of DFDBA to flap curettage with a reentry procedure between 6 and 13 months described significantly more bone repair (2.57 mm vs. 1.26 mm), more clinical attachment gain (2.91 mm vs. 1.53 mm), and less crestal resorption (0.47 mm vs. 1.20 mm) and gingival recession (0.19 mm vs. 1.33 mm) at DFDBA sites.[14] Seventy-eight percent of sites grafted with DFDBA had 50% or greater bone fill as compared to 40% for flap curettage.[14] For the eight defects in the present study treated with DFDBA, a decrease in probing depth of 3.1 ± 1.4 mm was noted along with a mean clinical attachment gain of 2.9 ± 1.6 mm. One year reentry measurements at DFDBA sites appeared to indicate minimal crestal resorption (-0.2 ± 2.1 mm), favorable apical defect fill (2.4 ± 3.1 mm), and greater than 70% defect resolution in 50% of the patients (defects) grafted.

Data in the present study were not normally distributed, necessitating nonparametric statistical analysis. Failure to reach statistical significance for any paired comparison of treatment groups over various clinical parameters evaluated in this study was primarily a function of the reduced size of the study population at the conclusion of the study. Although an initial design limiting this study to one positive control (DFDBA) would have increased the study sample to 16 and provided statistical power adequate for a parametric analysis, it was not possible at the outset to predict the healing response of sites treated with HAC relative to a specific control. Inclusion of both flap curettage and DFDBA controls provided two standards for comparison, both of which have been described in the periodontal literature as acceptable and successful treatment modalities with varying levels of therapeutic success. Based on the lack of information in the literature supporting the use of Hydroxyapatite Cement implant in the treatment of periodontal intrabony defects, investigators in the present study had no predetermined impression for how defects treated with this alloplastic implant cement would heal. Had only DFDBA controls been used, this study would have determined less favorable healing with HAC compared to DFDBA, but would not have allowed conclusions relative to the use of flap curettage. Inclusion of the flap curettage control group allowed the conclusion that clinically, HAC sites fared poorly relative to flap curettage controls.

Although nonparametric analysis revealed no significant differences in the soft and hard tissue healing parameters at sites treated with HAC compared to sites treated with flap curettage or DFDBA, a type II error working because of a small sample size likely prevents the detection of differences between test and control treatment modalities is this study. Descriptive information in the form of means for clinical hard and soft tissue parameters appear to indicate that both flap curettage and flap curettage plus DFDBA achieved a superior treatment outcome when compared to sites where defects were grafted with Hydroxyapatite Cement.

Clinical observations following implantation with HAC certainly indicate the material in its present form lacks utility in the treatment of periodontal vertical intrabony defects. Exfoliation and/or sequestration have been identified as the most common problems associated with osseous grafting.[90] Just as with osseous grafting, where immediate complete exfoliation of the graft with no improvement of the defect represents an extreme of failure for the regenerative attempt, complete exfoliation of the HAC implant represents failure of the implant to achieve clinical improvement in comparison to either flap curettage alone or flap curettage combined with a demineralized freeze-dried bone allograft. Unlike procedures where the degree of success is dependent upon how much of the total amount of an implant or graft exfoliates, complete exfoliation of the implant in this study appears to have served no purpose other than becoming an annoyance to the therapist and an inconvenience to the patient. Minor discomfort of the exfoliating implant combined with the increased difficulty for patients to perform plaque control procedures were findings that impacted negatively on the patient.

CADIA comparing baseline to 12 month postoperative standardized radiographs revealed statistically significant differences between HAC and both flap curettage and DFDBA at various areas-of-interest. In general, density gains at sites treated by flap curettage and DFDFA were contrasted by density loss at sites treated with HAC. Mean density loss in the apical third of defects treated with Hydroxyapatite Cement was contrasted by mean density gains by both DFDBA and flap curettage. Significantly different mean density changes were also noted at the middle and crestal thirds of the defect when sites treated with Hydroxyapatite Cement were compared to flap curettage. No statistically significant differences were noted when mean density changes for sites treated with DFDBA were compared to sites treated with flap curettage, although the greater increase in density at flap curettage sites approached statistical significance at AOI-1 ($p = 0.0209$) and AOI-4 ($p = 0.0820$). Mean density loss in the apical third of defects treated with HAC was contrasted by mean density gains by both DFDBA and flap curettage. Significant differences in density change were also noted in both the middle and crestal thirds of the defect when sites treated with HAC were compared to flap curettage. CADIA findings relative to density loss occurring at defects treated with HAC were consistent with clinical findings of pronounced resorption of bony walls in addition to actual loss of bone measured at the bony base of defects treated with HAC. Surprising findings of CADIA in this study included differences in mean density when density changes for sites treated with DFDBA were compared to sites treated with flap curettage. Mean density increases at the base of the defect were greater at flap curettage sites compared to sites treated with DFDBA. Although mean bony defect fill at sites treated with DFDBA suggests a clinically enhanced outcome at grafted sites, CADIA parameters suggest a more complex healing pattern relative to interpretation of density changes. Although a defect may experience actual bone fill within the defect and varying degrees of mineralization associated with that healing response, the bony walls of the defect may have experienced density loss or an eventual gain specific to the treatment modality of that defect. A trend associated with density change at sites treated with DFDBA was apparent as the area-of-interest moved coronally from the defect base into the middle-third of the bony defect. The mean density change for DFDBA sites at AOI-2 doubled relative to that observed at the base of the defect. Increases in density at the mid-defect level (or higher) would be desirable as formation of a bony cortex at a more coronal level within the defect would be reflected by a density increase by CADIA. This bony cortex was observed visually as well as in the subtraction image of several pairs of sequential radiographs at sites treated with DFDBA. However, density changes associated with the apical, middle and coronal thirds of the defect did not appear to correlate well with specific clinical hard tissue changes noted at reentry in this study. Superior mean density increases observed at the base of defects treated with flap curettage do not necessarily concur with clinical observations of bony changes. In comparison to clinical healing, increases in alveolar bone density appear to be delayed. Little information exists in the dental literature concerning mineralization and density changes of the healing alveolus so the time interval required for completion of these healing associated changes in unknown. Comparison of radiographic density change (as determined by C.A.D.I.A. at the radiographic apical and middle third of the defect) with apical defect fill (determined through reentry surgical measurement of the bony defect) in the present study revealed no significant correlation ($r^2 = .05$).

Future application of this material in periodontal regenerative treatment modalities would have to consider reformulation of the material in order to modify physical characteristics of the set cement. A softer final form of the set cement would be required to overcome the current deficiency of flexural resistance inherent to the hardness the material. Incorporation of a pore size into the material sufficient to support migration and ingrowth of cells, blood vessels and bone is almost certainly a necessity before future applications of this alloplast as a periodontal regenerative material are undertaken.[85,91,92,93,94] Based on the findings of this study, there is no rationale available to support the use of Hydroxyapatite Cement Implant as currently formulated for the repair and regeneration of human intrabony periodontal defects.

The limitations of this study should be considered when interpreting these results. There may be inherent weakness associated with a single examiner and clinical probing measurements. On the other hand, one study has shown approximately 90% intra- and inter-examiner agreement within ± 1 mm for attachment level measurements from a reference stent with a conventional manual probe.[95] Intra-examiner reproducibility within ± 1 mm averaging 97% for probing depth and 96% for attachment level measurments using a conventional probe was reported in another study.[96] In the same study, inter-examiner reproducibility (within ± 1 mm) averaged 98% for probing depth and 96% for attachment level measurements with a conventional probe.[96]

Customized acrylic occlusal stents with grooves at the desired probing locations were used in the present study for their provision of a fixed reference point apico-coronally and mesio-distally.[64] As the primary investigator accomplished both pre- and post-treatment measurements, inter-examiner variability was eliminated from this study. Intraexaminer reproducibility was evaluated prior to initiating the present study. When allowance for variability was included (± 1.0 mm variation between the first and second probings), overall reproducibility was 97.4% for probing depth and 93.7% for clinical attachment levels. Measurement of intrabony parameters during surgical procedures is most appropriately accomplished with one examiner for reproducibility as well as for expediency. While not well documented, less intra- and inter-examiner variability are associated with reentry measurements than with many other clinical measurements.[64] As the primary investigator in this study could not rely on the presence of an associate investigator, the single examiner limitation could not be overcome. A probing stent was utilized for all clinical soft and hard tissue measurements to reduce bias. Although a reproducibility pilot was accomplished to establish the reproducibility of the primary investigator, accuracy of probing for the single investigator cannot be determined.

The small study population in the present investigation limited the statistical power necessary to detect differences in the treatment modalities under investigation. Although the data accumulated in this study is clinically important and likely clinically significant, the statistical basis required to draw definite conclusions concerning the endpoints of this study was not obtained.

Summary

HAC in its present form lacks utility in the treatment of periodontal vertical intrabony defects. Clinical observations following implantation of vertical bony periodontal defects with HAC included exfoliation and/or sequestration. Exfoliation of the HAC implant with no improvement of the defect represents failure of the attempt to regenerate the periodontal defect. Exfoliation of the HAC implant resulted in failure of the implant to achieve clinical improvement in comparison to either flap curettage alone or flap curettage combined with a demineralized freeze-dried bone allograft. Clinical improvement at sites treated with DFDBA appeared to be superior to healing at sites treated by flap curettage alone, although the data upon which this conclusion is based are not statistically meaningful. Use of HAC in periodontal regenerative treatment requires considerable modification of the material in order to attain physical characteristics of the set cement consistent with osseointegration, including both flexural resistance and a pore size consistent with ingrowth of vascularized tissue. Although there is support for the use of Hydroxyapatite Cement Implant in the repair of craniofacial osseous defects, there is no rationale available to support the use of HAC as currently formulated for the repair and regeneration of human intrabony periodontal defects.

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PLATE 1. **Probe, probing stent and preparation of HAC for implantation.**

A. University of North Caroline-15 periodontal probe and custom fabricated acrylic probing stent. A lateral groove in the acrylic stent controlled probe placement into the deepest portion of the periodontal pocket and intrabony defect.

B. Set-up for mixing Hydroxyapatite Cement. Preparation of HAC for implantation included the incorporation of HAC powder with sterile water which were mixed on a glass mixing slab until a malleable "putty-like" consistency was achieved. In bony defects treated with HAC, the material was carried to the defect with a double ended amalgam carrier (Arnel Inc., Hempstead, NY.), condensed into the defect with an amalgam condenser (Ladmore #3, Hu-Friedy®, Chicago, IL), and contoured until the implant reached the height of the remaining osseous walls of the defect.

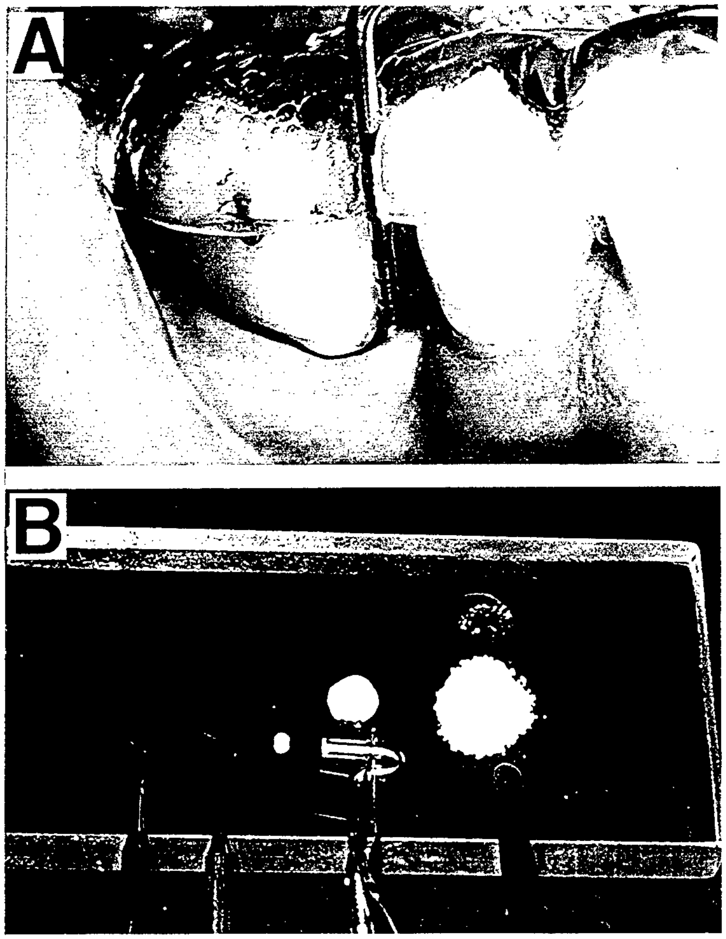
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PLATE 2. Treatment of a defect utilizing DFDBA including 12-month reentry.

A. Positive control (DFDBA) site. Bony defect following debridement with probing stent and UNC-15 probe positioned in the deepest portion of the bony defect on the mesial of the mandibular first molar.
B. DFDBA reconstituted with sterile water was placed to the height of the remaining osseous walls of grafted bony defects.
C. Pre-op radiograph of vertical bony defect on the mesial surface of the mandibular first molar.
D. Twelve-month post-operative radiograph taken prior to surgical reentry.
E. Clinical photograph of the mandibular first molar taken at 1-year immediately prior to surgical reentry.
F. Residual defect observed on reentry of the defect at the mesial of the first molar which was initially treated with DFDBA. The residual defect measured less than 1 mm in depth and was eliminated with an osseous resective treatment approach.

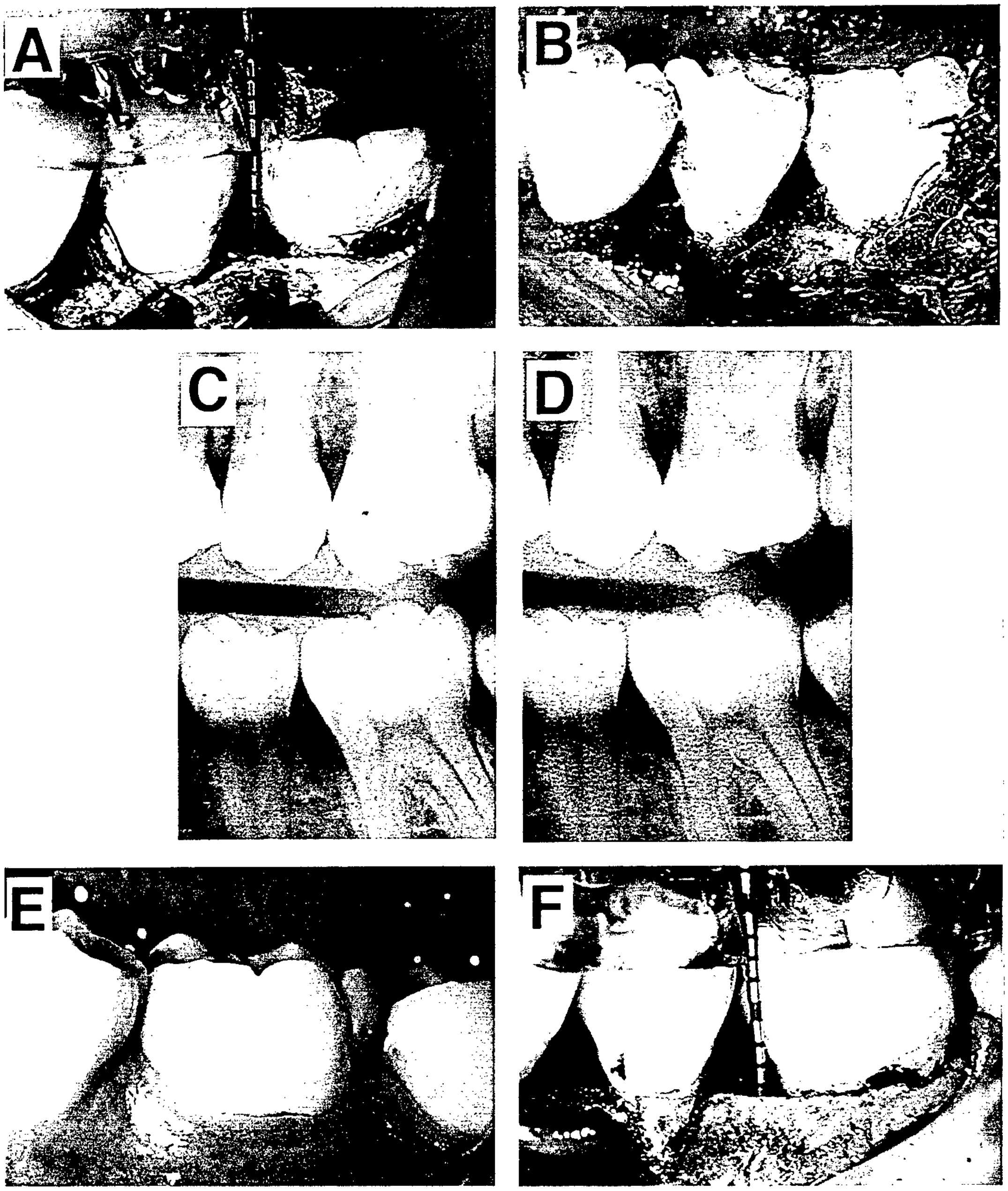
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PLATE 3. Treatment of a defect utilizing HAC implant.

A. Test (HAC) site. Bony defect following debridement prior to placement of Hydroxyapatite Cement implant.
B. Pre-operative radiograph of the vertical bony defect on the distal of tooth #19.
C. Defect following implantation of HAC to the alveolar crest, prior to flap closure.
D. Immediate post-operative radiograph of the HAC treated defect.

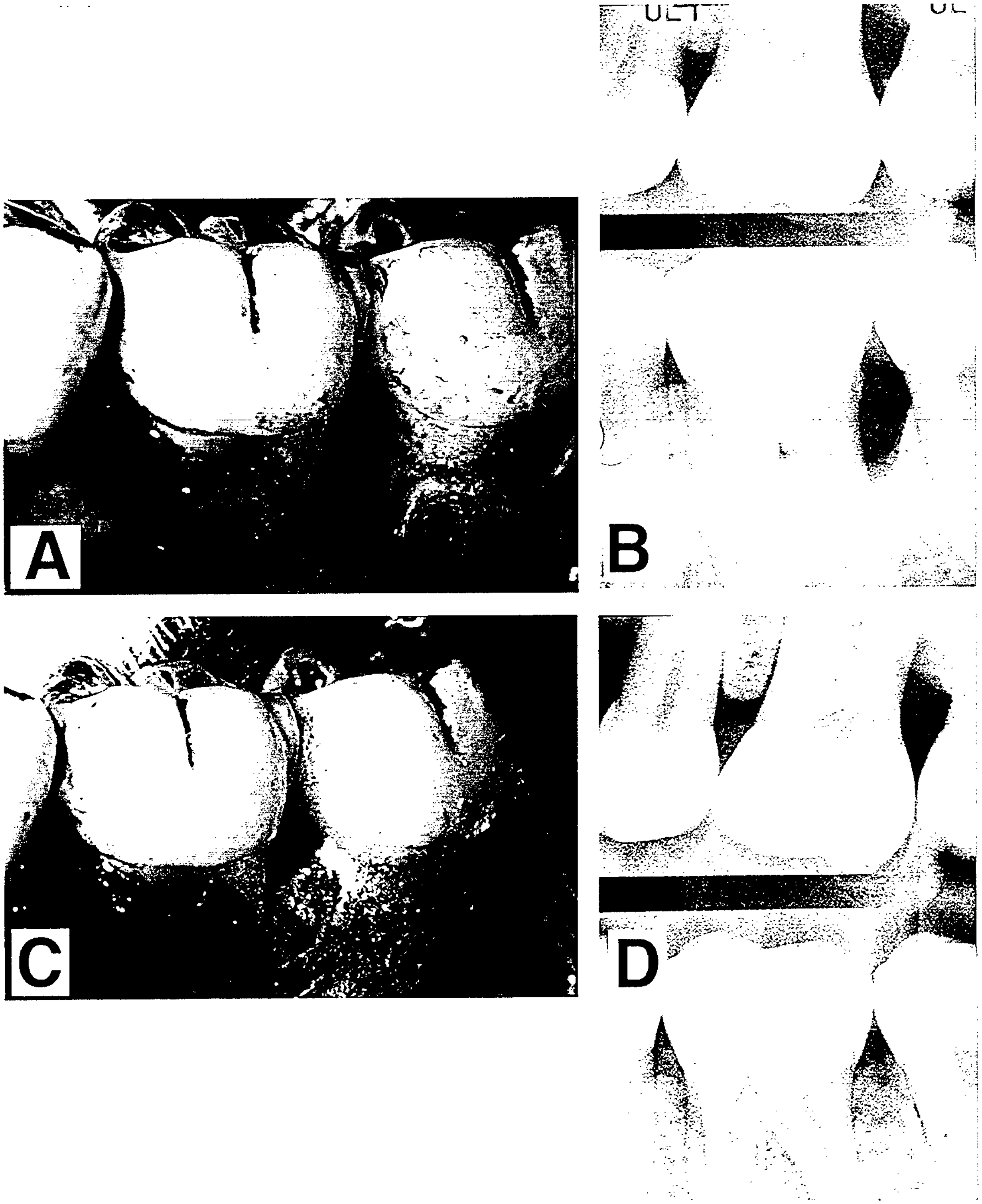
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PLATE 4. Treatment of a defect utilizing HAC implant including 12-month reentry.

A. Continuation of the sequence shown on plate 4 of the defect treated with HAC. This photo represents the clinical appearance of the treated area at one year following the initial surgical implantation of HAC but prior to reentry surgery.
B. Post-operative radiograph taken approximately 12 months following initial surgery. Residual HAC is visible in the coronal aspect of the defect.
C. Clinical reentry photograph following flap reflection. Residual HAC can be observed within the granulation tissue of the bony defect.
D. Residual defect following debridement. At the 12 month reentry, a defect was present at this site which was treated initially with HAC. The residual defect was treated with DFDBA and a Guidor® Matrix Barrier.

(Guidor® Matrix Barrier, John O. Butler Company, Chicago, IL)

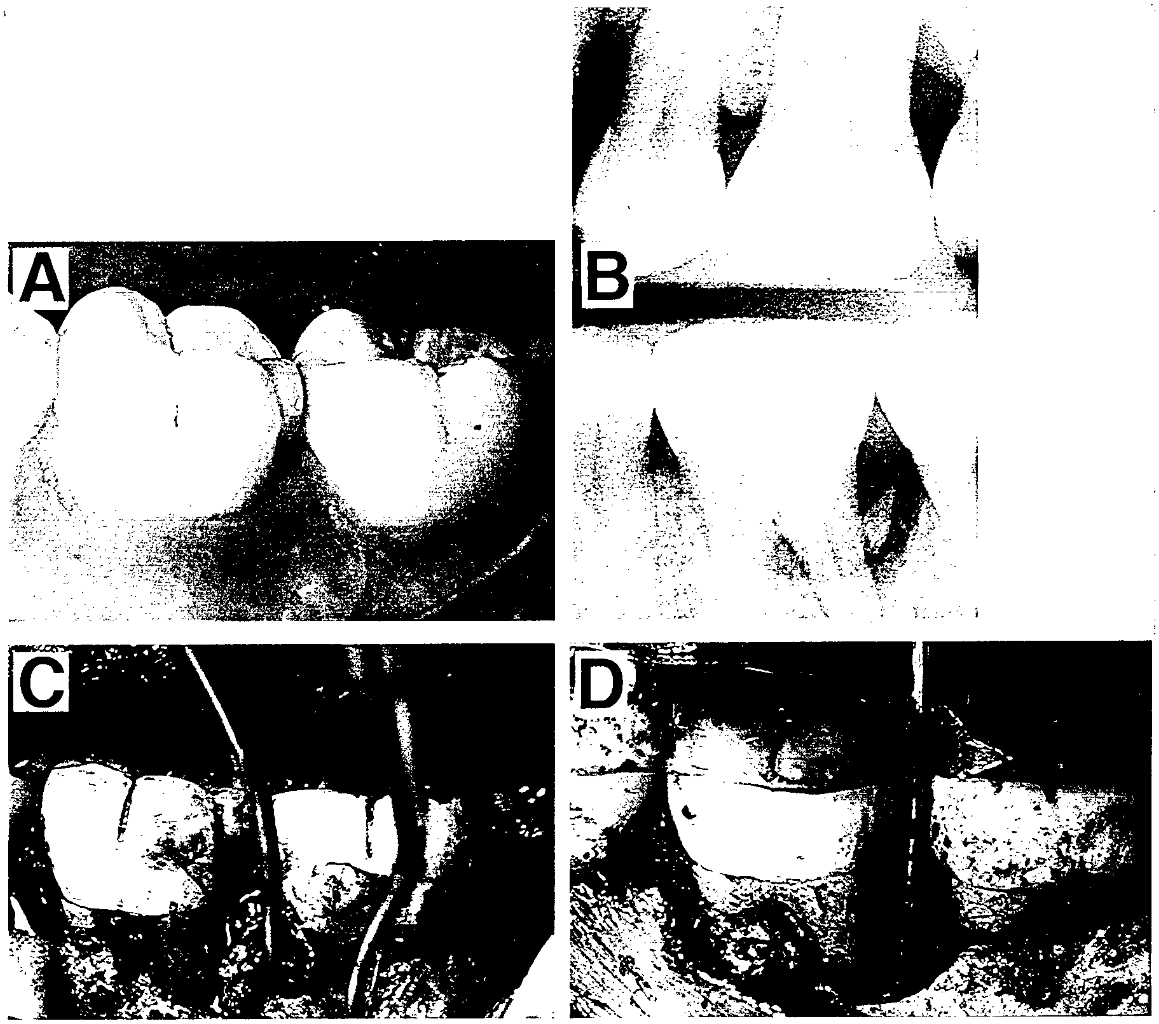
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PLATE 5. **Cone alignment device and customized film holder for standarized radiographs.**

A. Cone alignment device (Rinn Corporation, Elgin, IL) customized with three parallel guide pins allowed consistent and reproducable alignment of the x-ray cone perpendicular to the radiographic film. The bite registration material was added to the film holder in order to capture an occlusal registration.

B. Cone alignment device with customized film holder in place in patient's mouth.

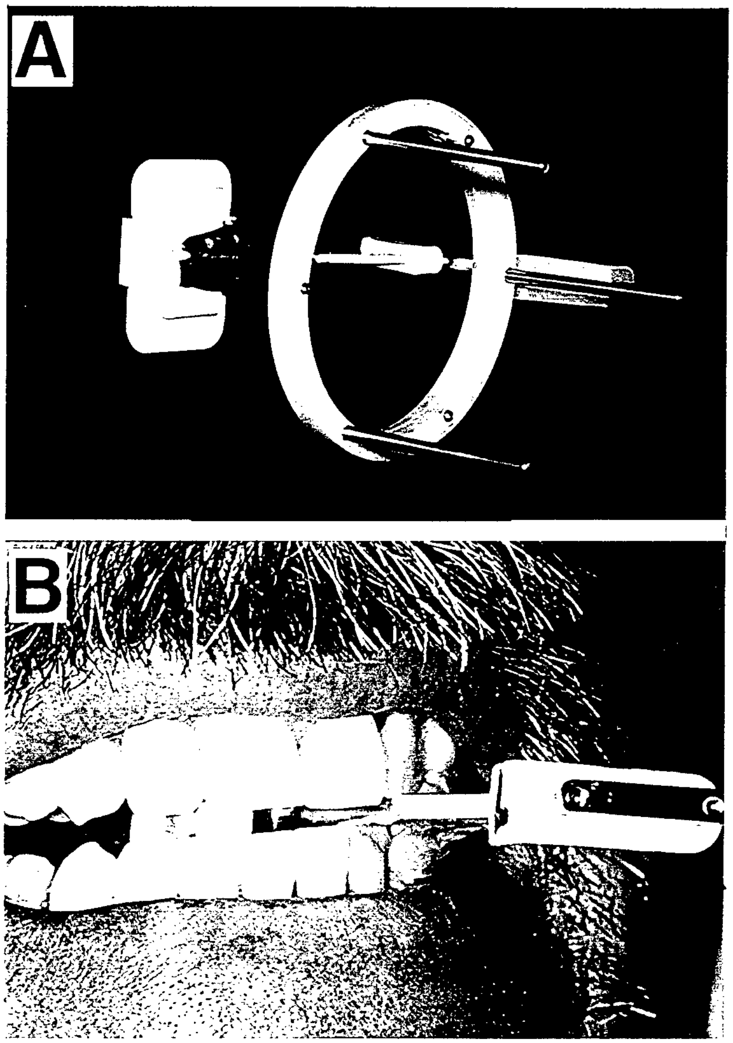
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PLATE 6. **Radiograph of a vertical bony defect with boxes representative of the areas-of-interest positioned for CADIA.**

Five areas-of-interest (AOIs) were selected for each intrabony defect using preformed 16 x 16 pixel boxes, each representing 0.4 mm^2 of area. AOI-1 was positioned in the apical third of the bony defect close to the visible base of the defect. AOI-2 was positioned in the middle third of the defect. AOI-3 was positioned approximately 1 mm apical to the alveolar crest in the coronal third of the defect. AOI-4 was positioned immediately apical to AOI-1 below the bony cortex of the defect's base or lateral wall. AOI-5 (not shown) was positioned in an area where no change was expected and for informational purposes only.

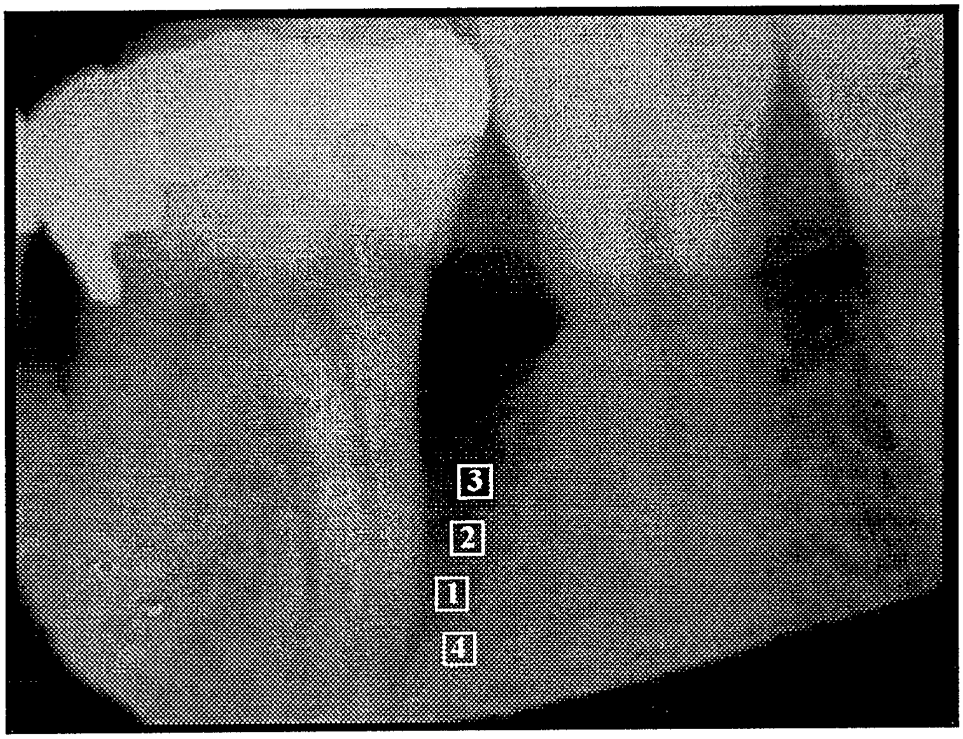
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PLATE 7. Subtraction image.

Images stored digitally for CADIA were retrieved for subtraction radiography and used as a subjective visual aid. This subtraction encorporates the pretreatment radiograph and the 12-month post-op radiograph exposed prior to reentry surgery. The lighter areas along the bony cortex of the defect (which appear white) are representative of increasing density.

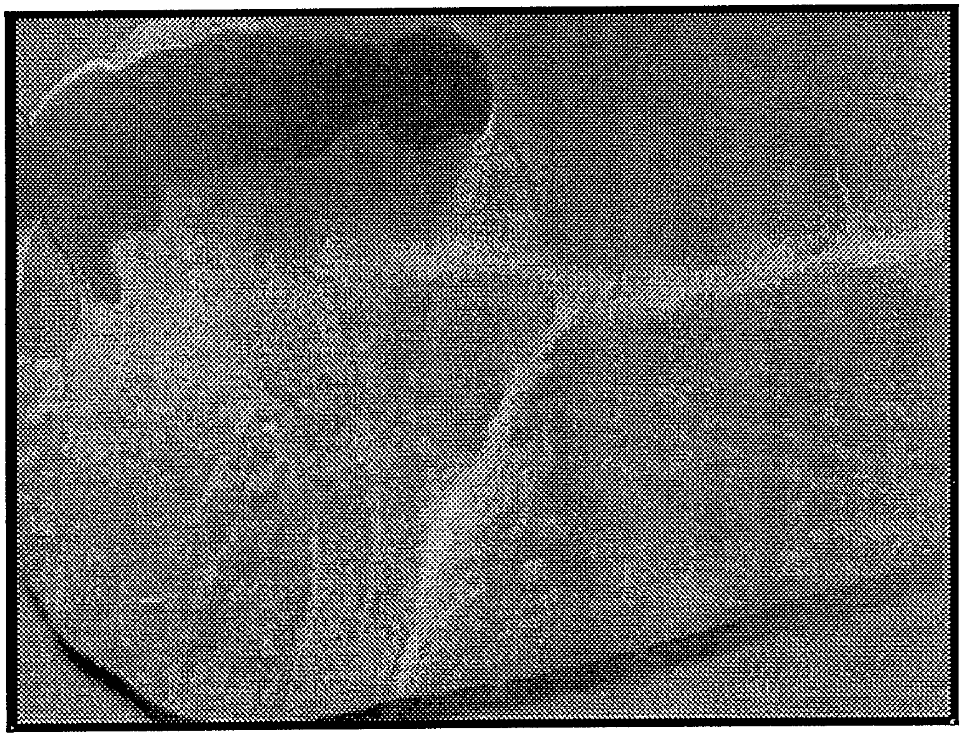

PLATE 8. Exfoliation/sequestration of HAC implant.

A. Test site. The defect on the mesial of the first molar was treated with HAC which exfoliated within one month after surgical placement.
B. Test site. The defect on the distal of the second molar was treated with HAC which exfoliated through the gingival sulcus approximately 3 weeks following surgical implantation.
C. Sequestered implant. In this photograph of an entire sequestered HAC implant, the positive impression of the depressions where intramarrow penetration was completed are present on the surface of the implant.
D. SEM photo of sequestered and freeze fractured HAC implant. In this SEM photomicrograph, an irregular surface with small micropores on the order of 4 to 10 μ can be appreciated (magnification x 1490 with each bar = 10 μ).

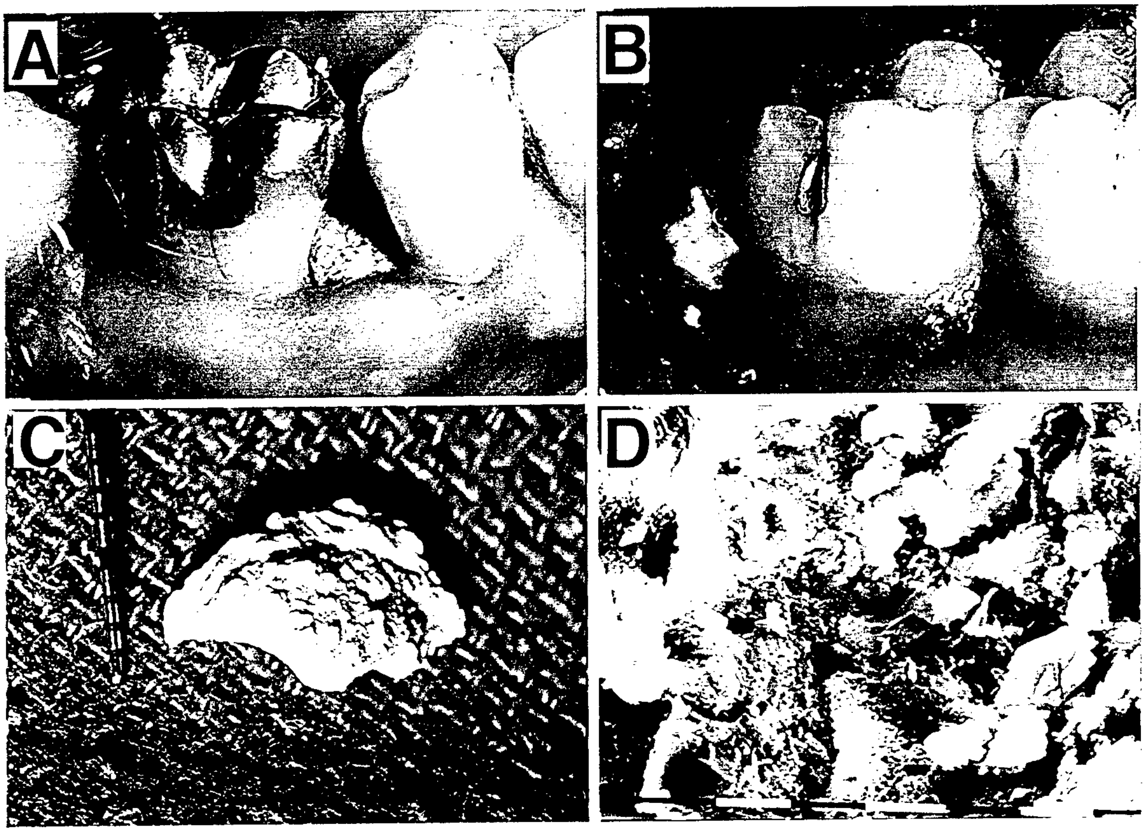
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PLATE 9. Radiographic appearance of a defect treated with HAC implant at various times postoperatively.

A. Pre-operative radiograph of tooth #19 demonstrating a vertical bony defect on the distal surface.

B. Immediate post-operative radiograph of tooth #19 demonstrating initial close adaptation of HAC to the bony walls of the defect.

C. Two-week post-operative radiograph of the defect demonstrating the formation of a radiolucent gap.

D. Twelve-month pre-reentry radiograph of the defect where HAC had previously exfoliated. Visual impression that the defect has increased in depth compared to the pre-operative appearance. This defect was treated with a Guidor® Matrix Barrier and DFDBA following debridement at reentry.

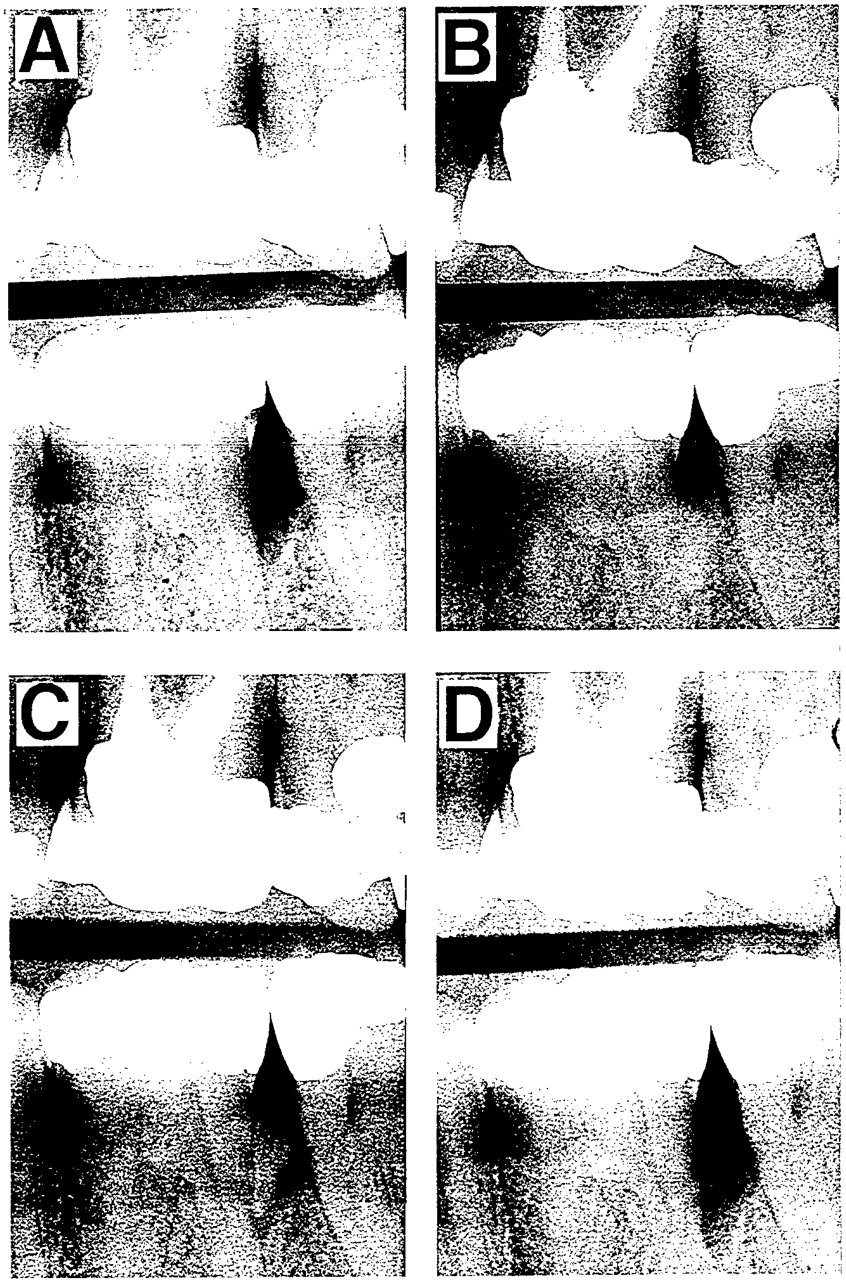
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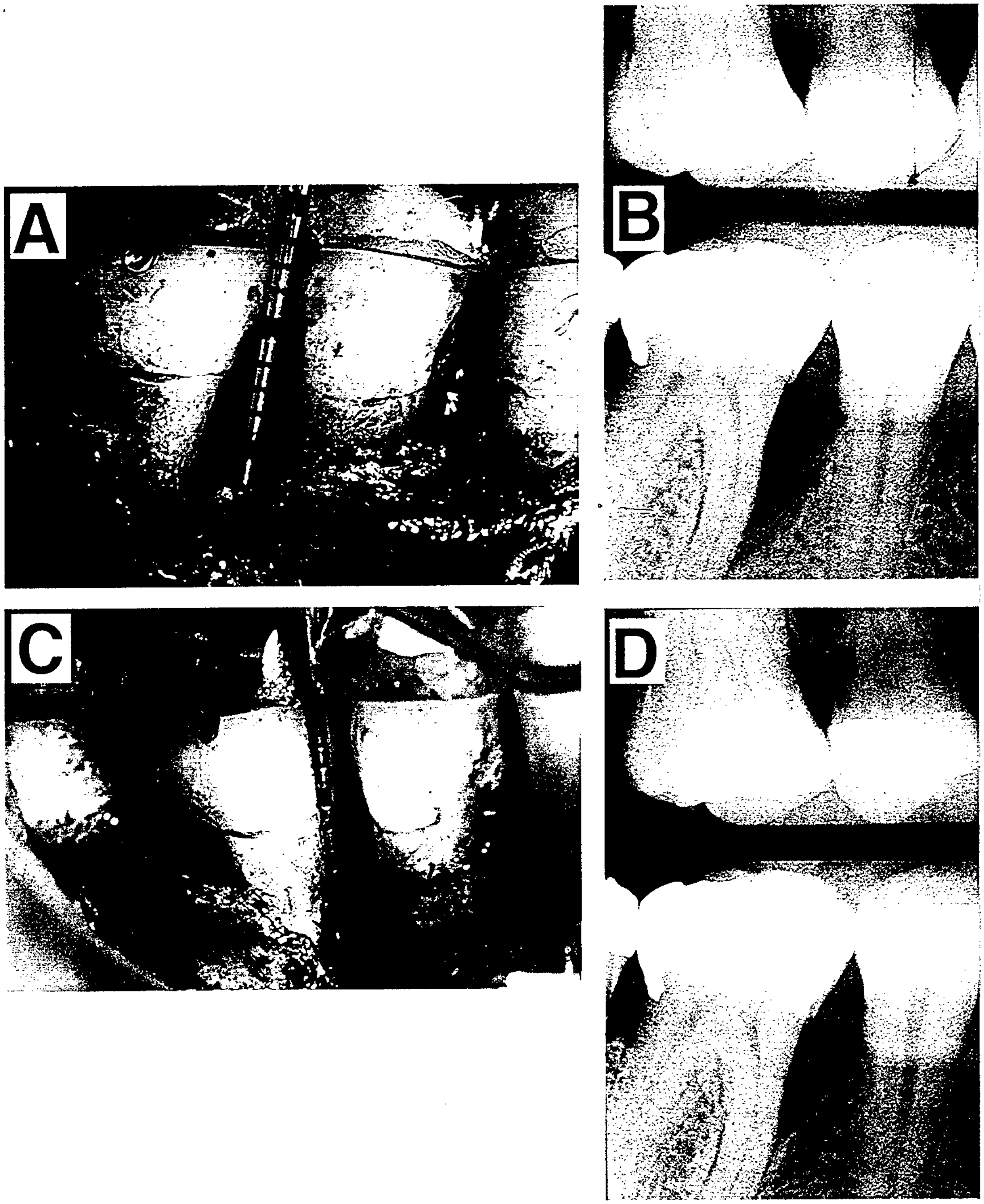
PLATE 10. **Treatment of a defect utilizing flap curettage including 12-month reentry.**

A. Negative control (flap curettage) site. Bony defect following debridement prior to flap repositioning and closure.
B. Pre-op radiograph of vertical bony defect on the mesial surface of the mandibular first molar.
C. Residual defect observed at reentry of site treated with flap curettage only. The residual defect was treated with a Guidor® Matrix Barrier and DFDBA following thorough debridement.
D. Twelve-month post-operative radiograph taken prior to surgical reentry.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VITA

Graig D. Brown was born in Des Moines, Iowa on 17 August 1958 to Rodney and Wanda Brown. Following two years of undergraduate training at Culver-Stockton College in Canton, Missouri, he transferred to Drake University in Des Moines where he graduated magna cum laude on 17 May 1980. During his undergraduate years, he was elected to Alpha Epsilon Delta national premedical honor society and Beta Beta Beta national biology honor society. On 12 May 1984, Graig received his Doctor of Dental Surgery degree from The University of Iowa, Iowa City, Iowa. Dr. Brown was commissioned in 1984 as an officer in the United States Air Force and proceeded to Davis-Monthan Air Force Base in Tucson, Arizona where he assumed the duties of general dentistry officer. After 5 years on staff at the USAF Hospital at Davis-Monthan, he was reassigned to the USAF Clinic, NATO Air Base Geilenkirchen, Germany in June of 1989. While serving a three year tour at Geilenkirchen as a general dentistry officer, Dr. Brown also served as Assistant Base Dental Surgeon. Following a one-year assignment to the Base Dental Service at Lackland Air Force Base Dunn Clinic, Dr. Brown entered graduate training in Periodontics in June of 1993 at Wilford Hall Medical Center and the University of Texas Health Science Center at San Antonio. Dr. Brown married his dental school sweatheart Miss Jean Marie Devitt on 17 July 1982 in LaGrange, Illinois. They have two sons, Jonathan Devitt Brown and Joshua Devitt Brown.